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MICROBIOLOGY, 1776-1908

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MICROBIOLOGY

HISTORICAL CONTRIBUTIONS
FROM 1776 TO 1908 BY

Spallanzani, Schwann, Pasteur, Cohn, Tyndall,
Koch, Lister, Schloesing, Burrill,
Ehrlich, Winogradsky, Warington,
Beijerinck, Smith,
Orla-Jensen

Edited by Raymond N. Doetsch

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PREFACE

The selection of papers for a collection of historical contributions to microbiology naturally involves personal choice and bias, and perhaps some explanation is in order. In general, the papers in this volume reveal important historical antecedents of microbiology before it was arbitrarily divided into a number of specialties, as, for example, industrial microbiology, agricultural microbiology, and biochemical microbiology. It is hoped that a perusal of this collection will enable the reader to view microbiology as a whole and, further, to trace the beginnings of the narrow specialisms of the present day. Very often students of microbiology become so involved in being "trained" that they (and their professors) fail to realize that the science did not spring into existence "fully armed as Pallas Athena from the head of Zeus." The history of science is concerned with ideas, how they evolved and are logically related, and the conditions under which they were generated. Clearly, one whose knowledge of the development or logic of an idea is nonexistent, or ill-conceived, can hardly be presumed to understand it.

A study of original contributions in science may reveal a number of interesting points to the perspicacious reader. Direct contact with great original works can provide a powerful stimulus to the scientific imagination; but whereas some may look upon historical studies of science as mere antiquarianism, others may delight in following the checkered path of progress unblushingly uncovered for all to see. This collection may serve, therefore, both as material for a course in the history of general microbiology and as a supplementary source for the study of a relatively poorly documented branch of biology.

Assembled here are original accounts of contributions made to microbiology over a period of one hundred thirty-two years (1776 to 1908). The scientists represent Denmark, France, Germany, Italy, the Netherlands, the United Kingdom, and the United States. The sequence of papers is, in the main, chronological, but the development of microbiology as a science may be discerned. One proceeds from the question of the origin of microbes (Spallanzani, Schwann, Tyndall), to their role in fermentations (Schwann, Pasteur), to methods for dealing with them in the laboratory (Koch, Ehrlich) and classifying

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them (Cohn, Orla-Jensen), to a recognition of the importance of their activities as biological entities (Lister, Schloesing, Warington, Winogradsky, Burrill, Smith, Beijerinck). Most of the papers are from journals relatively inaccessible to the modern reader, and a number have not been previously translated into English.

The papers of Spallanzani, Schwann, Pasteur, Tyndall, and Lister illustrate their brilliant efforts in coping with the ancient problem concerning the origin of those living things whose mode of reproduction could not be directly apprehended. It is enlightening to note the variety of backgrounds from which these men attacked the problem, that is, from the point of view of a naturalist, a physiologist, a chemist, a physicist, and a surgeon. The benefits accrued from their contributions were far more important than their ultimate verdict that living beings could not be generated *de novo* under the experimental conditions designated by the proponents of the doctrine of abiogenesis. In essence, much of the technical foundation of the science of microbiology was established, and concomitantly many of the biological properties of microbes were revealed. Only after these developments occurred could the rational control of the activities of microbes be attempted.

The two contributions of Koch contained in this book reveal him as the master of bacteriological technique. Many of Koch's methods have survived to this day in but slightly modified form. It is true, of course, that Koch did not work alone, and his school included many gifted and highly original workers who were overshadowed by Koch's brilliance. Among them are W. Hesse, R. J. Petri, F. Loeffler, G. Gaffky, and G. Wolffhügel. Among the solid contributions of Koch and his school to techniques were vastly improved methods of staining, photographing, and cultivating bacteria. It was through his perfection of the agar plate method that Koch opened the way for the almost unbelievable advances in the conquest of diseases caused by bacteria. The development of this technique also marked the beginning of microbiology as a quantitative science.

Ehrlich introduced methylene blue into bacteriological work in 1881. It was widely used thereafter and formed the basis of F. Loeffler's alkaline methylene blue, developed in 1884, and used to the present day in microbiological laboratories. Ehrlich also improved upon Koch's method for staining *Mycobacterium tuberculosis* by using saturated gentian violet or fuchsin solutions in aniline water. This technique constituted the essentials of the so-called "acid-fast" stain, in that once the tuberculosis microbe was dyed, it could not be decolorized with 30 per cent nitric acid. In brief, Ehrlich developed the art of bacterial staining by sound practical and theoretical contributions to the point where it was no longer a set of empirical operations, but a truly scientific process.

Two pioneers of American bacteriology, Burrill and Smith, are

also represented here. It is a curious development in the history of microbiology that many of the arguments used *contra* microbes as possible agents of human and animal disease were again employed when it was found that they were also involved as phytopathological agents. In fact, "rust" of wheat was postulated to be caused by fungi as early as 1813. In spite of this, there was a tendency to consider such environmental conditions as rain, cold, and external trauma as primary factors predisposing the plants to secondary attack by microbes. This view is reminiscent of the doctrine promulgated by R. Virchow concerning human disease, that is, pathological changes were the result of physiological transformations, and the true cause of disease resided in the tissue cells themselves. It is to the credit of Burrill and, to an even larger extent, of Smith that they established, defended, and extended the principles discovered by Pasteur and Koch to the plant world.

Schloesing and Müntz demonstrated, in a classical experiment, that the microbes of the soil were capable of bringing about the oxidation of ammonia to nitrate. Since this is a process of vital importance for the maintenance of life on earth, it is not surprising to learn that a number of investigators attempted to isolate microbes with this ability. The work of Warington shows clearly the great difficulties involved, and the numerous pitfalls that beset individuals working on the problem. It remained for Winogradsky to provide the key to its solution. Prior to this work, Winogradsky developed the concept of chemolithotrophy (autotrophy) from his investigations on filamentous, colorless sulfur bacteria. He showed that these microbes derived their energy from the oxidation of a purely *inorganic* compound (hydrogen sulfide), and that elemental sulfur was deposited within their cells. In the absence of a continued hydrogen sulfide supply, the deposited sulfur was oxidized to sulfate. This work at once revealed the far-reaching biochemical potentialities of the microbial cell, and also demonstrated a hitherto unrealized mode by which it derived energy for growth and synthesis. Armed with this experience, Winogradsky reasoned that nitrification might also be mediated by autotrophic microbes. He thereupon provided microbiology with a technique worthy of Koch himself, namely that of cultivating the desired organisms on a purely inorganic medium. In this classical work he isolated the long-sought-for nitrifying bacteria. A reading of Winogradsky's paper will reveal the fascinating working of his mind on this, an almost "insoluble" problem.

In the three papers of Beijerinck one may read how he developed a medium favoring the growth of an important sulfate-reducing organism. This method, known as the "enrichment culture technique" inaugurated by Winogradsky, and further refined by Beijerinck, had far-reaching consequences for microbiology. Microbes possessing a desired physiological property could be obtained by defining the

conditions (carbon and nitrogen source, pH, etc.) of the medium, and then inoculating it with the mixed microbial flora from any source. Those organisms capable of proliferating under the predetermined conditions would be selected out or "enriched," whereas the remainder of the flora would be largely inactive. Using such manipulations, microbiologists could now isolate and study microbes with any desired characteristic, provided they existed in nature. The "enrichment culture technique" has proved to be one of the microbiologist's most rewarding methods.

Orla-Jensen's influence is strongly felt today by bacterial taxonomists. His classification scheme, the first to be based primarily, but not exclusively, on physiological (as contrasted to morphological) properties, was evolved from a logical development of his ideas concerning the course of evolution of life on earth. In this system, microorganisms were assumed to have evolved from chemosynthetic (autotrophic) to chemoanalytic (heterotrophic) forms possessing increasingly complex nutritional requirements. At the same time, a new system of nomenclature was introduced in which the organisms were assigned names suggesting their physiological capabilities as well as their morphology. This attempt at a "natural system" of classification, reflecting phylogenetic development, has exerted a great influence on modern systems despite many criticisms made against it. At any rate, after Orla-Jensen's proposals were published, physiological properties were used as unhesitatingly as morphological features in classification (or identification) schemes.

Here then, is a record of the thoughts and work of "other men in other times." Obviously, the work of today will one day also be described in these terms. It is hoped that a perusal of this collection will reveal something from the past generations of microbiologists that is essentially unaltered by the passage of time.

The introductory notes of each chapter are intended merely to suggest some reasons for the importance of the contribution under consideration. It is hoped that the interested reader will attempt to satisfy himself concerning the "strategy and tactics" used in attacking the problem with which each paper is concerned. The references supplied for further reading are included as convenient, though not exhaustive, sources of background material (mostly in English) for a more detailed study. In a few instances, sections of very long papers have been deleted, but in the main these sections did not contain illustrations of new principles; and in each case a short note is given summarizing the deleted material.

Many persons gave generously of their time and knowledge in making this venture a success. The editor would like particularly to express his deep-felt appreciation to Vernon Bryson, associate director, Institute of Microbiology, Rutgers University, for his encouragement of this project; to Miss Ellen Mayeux, reference librarian of the

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Raymond N. Doetsch

January 4, 1960
College Park, Maryland

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MICROBIOLOGY, 1776-1908

I.

LAZZARO SPALLANZANI

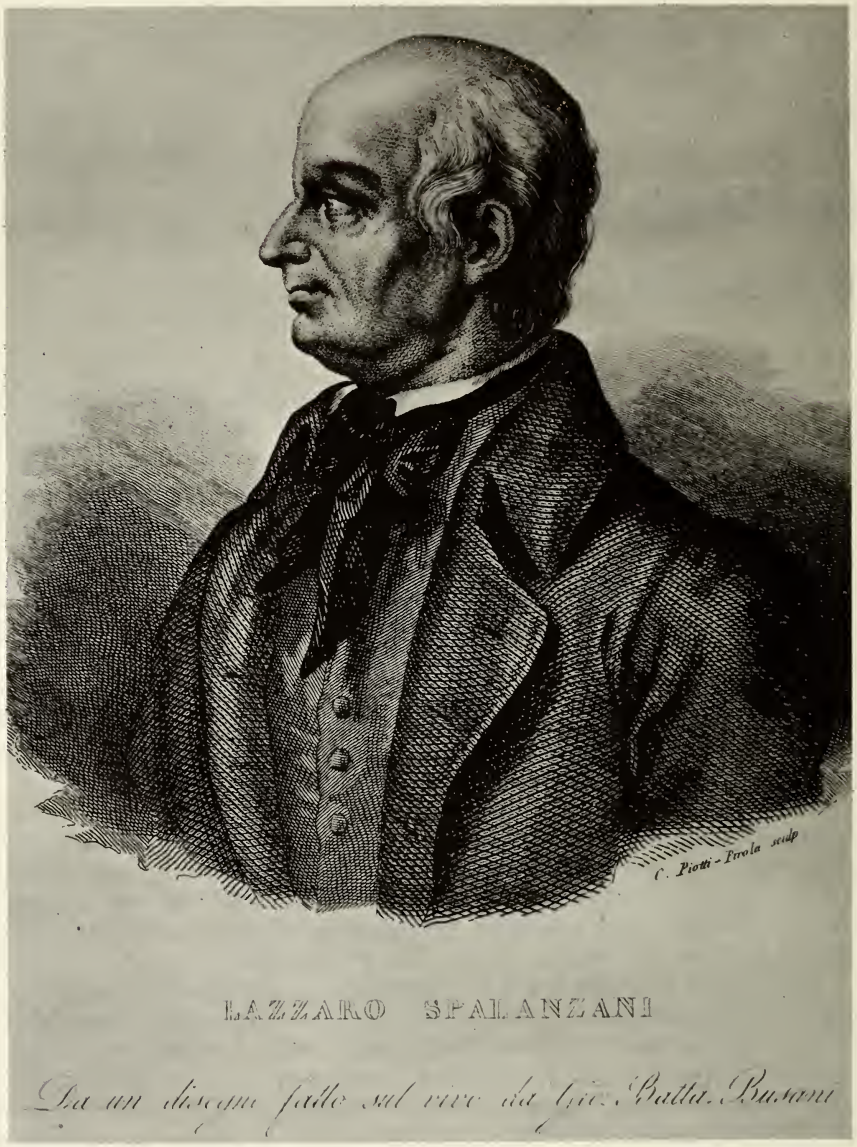
(1729 to 1799)

Lazzaro Spallanzani was born at Scandiano, near Modena, Italy, on January 10th, 1729. In 1747, after attending the Jesuit College at Reggio, he entered the University of Bologna, with the intention of becoming a lawyer, like his father. Soon, however, under the influence of his cousin, Laura Bassi, a professor of physics at Bologna, he turned his attention to mathematics and physics. At the age of twenty-six, he became a priest. He returned to Reggio as professor of logic and metaphysics in 1755, and eight years later moved to a post at the University of Modena. In 1769, he was appointed to the chair of natural history at Pavia, and there he remained until his death, on February 12th, 1799. Under the sponsorship of Queen Maria Theresa, he initiated zoology classes and devoted great effort to the acquisition of books, equipment, and scientific collections. He was responsible for the establishment of the Museum of Natural History at Pavia.

A keen and precise experimenter, Spallanzani possessed an instinctive appreciation of scientific principles. In many respects, his work was far in advance of the times, and it is gratifying to note that a true understanding of his genius has now emerged. He was a member of academic and scientific societies in Bologna and Siena, and also in London, Stockholm, Berlin, and Montpellier.

Spallanzani's greatest work, on processes of digestion and reproduction, was published in 1776 under the title *Opuscoli di Fisica Animale, e Vegetabile.* He concerned himself with a wide range of biological problems including digestion processes after death, artificial insemination of dogs, measurements of blood velocity, desiccation of rotifers, cutaneous respiration, and the nature of infusoria. Although his principal contributions were in the field of experimental physiology, he was active in the fields of physics, geology, and mineralogy, and made a number of investigations on volcanoes and mines.

In connection with his studies on the anatomy and physiology of infusoria, he became involved in the famous controversy with J. T.



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Lazzaro Spallanzani

Needham and G. L. LeClerc (Comte de Buffon) concerning spontaneous generation. His work on this problem is detailed in his *Saggio di Osservazioni Microscopiche Concernenti il Sistema della Generazioni dei Signori Needham e Buffon,* published in 1765. Although Spallanzani was unable to perform the crucial experiment *contra* abiogenesis, his work clearly demonstrated the effects of various physical agencies on infusoria, and some of the reasons for their appearance in heated infusions. Spallanzani knew that in order to prevent infusions from producing infusoria it was necessary to heat them at 100° C. for at least forty-five minutes. Needham had objected that such an exposure to heat destroyed the elasticity of the air and destroyed the vegetative force of the infusions. Spallanzani did not analyze the air of the infusions (indeed the methods for doing so were not yet devised), nor could he devise any method whereby unheated air could be led into infusions without leading to the development of animalcules. His conclusions regarding spontaneous generation were, nevertheless, almost identical with those reached by Louis Pasteur nearly a century later.

Spallanzani possessed no real knowledge of bacteria, and his morphological descriptions of the organisms seen by him in infusions are useless for identification purposes. Yet he began the experimental attack against proponents of the doctrine of spontaneous generation of animalcules (although L. Joblot initiated experimental work along similar lines as early as 1710), and his work served as an example for numerous investigations by the many workers who followed on both sides of the controversy.

The translation, below, is adapted slightly from the one made by John Graham Dalyell in *Tracts on the Nature of Animals and Vegetables,* published in two volumes in 1799 by Creech and Constable in Edinburgh. Chapter I of Part One of this translation is presented below. The original work was published in Modena in 1776 as "Osservazioni e Sperienze Intorno agli Animalucci delle Infusioni" in Spallanzani's book *Opuscoli di Fisica Animale, e Vegetabile.*

OBSERVATIONS AND EXPERIMENTS ON THE ANIMALCULA OF INFUSIONS

Whether, According to a New Theory of Generation, Animalcula Are Produced by a Vegetative Power in Matter: Infusions and Infused Substances Exposed to Heat.

Nothing is more common with philosophers who have invented any theory, or given a new form to one already established, and universally known, than to republish it on some other occasion, corrected, improved, or illustrated, with additional information. If we would review our discoveries, if we would examine them profoundly and with impartiality, we should, in general, find defects unnoticed before, which arise from the want of connection in sentiment, from the want of a necessary and laudable perspicuity, or because they are discordant with more recent discoveries.

A certain vegetative power some have conceived to reside in matter, appropriated to the formation and regulation of organized existence. By it are the numberless combinations of the animal machine effected: the operation of nutrition and perspiration, the variety of constitution, the animal appetites and dimensions of the human frame. By the same means has it been explained why a blind or maimed person may have children vigorous and entire: because the vegetative power will restore to them the members defective in the parent.

Not only has vegetative power been supposed to be requisite for the organization of matter in animated beings, but that it might change an animal to the vegetable state, and the vegetable again to an animal; that it acts on plants while living, and when dead, regenerates them in new beings. These are the animalcula of infusions, which cannot strictly be called animals, but beings simply *vital.*

One proof adduced in support of this hypothesis is derived from the origin of animalcula. We are told that they must either come from specific seeds or be produced by the vegetative power; that the first cannot take place, because they are found in closed vessels subjected to the action of heat, as well as in open vessels, whereas the included germs, if there were any, ought not to survive. Therefore, they must originate from the vegetative power alone. Nothing has been omitted to obtain favorable arguments for this opinion, and to give it that clearness, elegance, and simplicity most likely to gain converts.

Nineteen vessels containing infused substances were hermetically sealed, and kept an hour in boiling water. Being opened at a proper time, not a single animalculum was to be seen. To this experiment of mine, it was objected that the long continuance of heat had perhaps entirely destroyed the vegetative power of the infused substances,

or materially injured the elasticity of the air remaining in the vessels; thus, it was not surprising if animalcula did not appear.

To eliminate the weight of these objections, I conceived an experiment, apparently decisive, which was to make nineteen infusions, and boil some of them a short time, others longer, and the rest very long. If the vegetative power of infused substances was destroyed, the numbers of animalcula would be less according to the duration of boiling; if not, the number would be alike in all cases.

Vegetable seeds, being the most fit for producing animalcula, were preferred to other substances, and never failed to produce them though they had experienced the effect of heat. White kidney beans, vetches, buckwheat, barley, maize, the seeds of mallows and beets, were infused; and, that the experiment might be more accurate, I endeavored as much as possible to take each species of seed from the same plant. As the yolk of an egg in maceration abounds with animalcula, one was also infused.

By experiment, I have demonstrated that the heat of boiling water is not always the same, but greater if the atmosphere is heavier, and less, if lighter; therefore, water will acquire more heat at one time than another, in accordance with the state of the atmosphere. In this, and my previous experiments, the seven different kinds of seeds, and the yolk, were all boiled an equal time, that they might acquire the same degree of heat. Here the experiment was diversified, by boiling a certain quantity of each infusion half an hour; another quantity, an hour; a third, an hour and a half; and a fourth, two hours. Thus, four classes of infusion, and the egg, could be formed. The same water, in which the seeds had boiled, was taken for the infusions, and what had boiled half an hour alone taken from the seeds that had boiled half an hour. The like proportions of time were preserved in the water for the other three classes of infusions; that is, an hour, one and a half, and two hours.

Each of the four classes was marked with a different number, to avoid all hazard of confusion or error; and because an equal temperature was most essential, all were deposited in the same place. The vessels containing the infusions were not hermetically sealed, but loosely stopped with corks, the only object of this examination being to discover whether long-protracted ebullition would prejudice or destroy the property of infused substances in producing animalcula; if it did, there would be no difference whether the vessels were open or closed.

The examination of one or a few drops will often induce an observer to suppose the infusion quite deserted, or very thinly inhabited, whereas the observation of many drops proves it to be otherwise. I was not content with one drop only, but uniformly took a considerable number from each infusion.

The surface of infusions is generally covered with a gelatinous

scum, thin at first, and easily broken, which, in process of time, acquires consistence. Here, animalcula are always most numerous, as may be seen by a method I have constantly practiced, examining with a magnifier a portion placed in a strong light.

Where the animalcula are minute, or rare, the thickness of the infusion often prevents the observer from distinguishing whether any are there or not. It is then necessary to dilute the drops with water. Elsewhere it has been remarked that distilled water was taken to make the infusions; common water might introduce some latent animalculum. In the course of these observations and experiments, distilled water was also employed for dilution, when required; and, for greater security, it was examined with a magnifier before being used. In particular cases, the accidental concealment of a single animalculum might vitiate the truth of the experiment.

I conceive it my duty to mention precautions so essential, and to put it in every individual's power to judge not only of the experiments and observations themselves but also of the mode of conducting them in matters so delicate and important.

On the 15th of September, I made thirty-two infusions; and on the 23d, I examined them for the first time. Animalcula were in all; but the number and species differed in each. In the maize infusions, they were smaller, and fewer, the longer the duration of boiling. From this it may seem that although long-continued heat had not prevented the production of animalcula, it had contributed to diminish the number, or alter the kind.

But with the rest of the infusions it was otherwise: The kidney beans, vetches, barley, and mallow seeds were in a better condition after undergoing the violent effect of heat for two hours than those that had been exposed for less time. Let us go into the detail the subject merits.

In the infusion of kidney beans, boiled two hours, were three species of animalcula: very large, middle-sized, and very small. The first were partly umbellated in shape and attached to long filaments dragged along in their progress; the second were cylindrical; and the third globular. All three were incredibly numerous.

In the infusion of kidney beans boiled two hours were animalcula of the largest and smallest class, but few in number; still fewer in that boiled an hour; and fewest of all in that boiled half an hour.

The infusion of mallows that had been boiled two hours produced middle-sized circular animalcula, and also some very large, with the head extremely hooked. In two infusions boiled an hour, and an hour and a half, the number and species were the same; and though surpassed by those of the infusions boiled two hours, still they were much more numerous than those boiled half an hour.

In vetches boiled half an hour was an immense number of semicircular bell-shaped animalcula, all of considerable size, while in

those boiled an hour and a half, they were small and rare. Some bell-shaped animalcula might be seen in an infusion boiled an hour, but it gave the eye pain to discover a few; and these were most minute when it had boiled only half an hour.*

Those in a barley infusion boiled two hours were numerous beyond description, and large; part were of an elliptical shape, others oblong.† The infusions boiled an hour and a half had but a moderate number of animalcula, very minute, and some appeared when boiled half an hour.

There was no fixed rule with the remaining infusions. In buckwheat boiled an hour and a half were many more animalcula than in any other infusions of it. This also happened in the egg and beet seed boiled an hour; but it is to be remarked that fewer animalcula were in these two infusions boiled half an hour than in any of the rest.

Hitherto, the shape of these legions of animalcula has been cursorily alluded to. A circumstantial account is in my *Dissertations*, and it will be spoken of more at length in the course of this tract.

Thus, it is clearly evident that long boiling of seed infusions does not prevent the production of animalcula; and, notwithstanding the evidence that long boiling of maize does not seem to favor it, four other infusions strongly corroborate the fact.

Why is it that infusions boiled the shortest time have fewest animalcula? I cannot think myself mistaken in assigning the following reason. For animalcula to appear, it is necessary that the macerating substances give some indication of the dissolution of their parts; and, in proportion as dissolution advances, at least for a limited time, the number of animalcula will increase. The uniformity of this has been shown in another place, and would be confirmed, was it requisite, in these new inquiries. Now, if seeds have boiled a short time, they are less invested and penetrated by the dissolving power of heat; therefore, when set apart to macerate, they are not so soon decomposed as those longer boiled. Thus, there is no occasion for surprise if some infusions swarm with animalcula, whereas others have very few: And this I do believe to be the reason why, when two infusions are made at the same time, one of unboiled, the other of boiled seeds, animalcula are frequently observed much sooner in the latter than in the former.

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*Two classes of infusoria were distinguished by Spallanzani; one, the *ordini superiori*, was killed by boiling for half a minute, the other class, *ultime ordine*, was much more resistant, and occasionally survived boiling for half an hour. This class was much smaller microscopically than the first mentioned and perhaps contained bacterial representatives. *Editor.*

†Probably the author means different kinds of ellipse. At the same time, there are animalcula, though few, nearly of an oblong figure. Most of the descriptions in all this tract are so general, that it is difficult to ascertain the exact species of animalcula. *Translator.*

A little boiling will not decompose vegetable seeds, for decomposition is effected by flow and gradual maceration.

Some days after these experiments were begun, the number of animalcula became even greater, and towards the middle of October increased so much that the thirty-two infusions were equally swarming. The only difference was in size, shape, and motion. I enjoyed this pleasing microscopic scene uninterrupted until the 10th of November; and it might have amused me longer had I continued to examine the infusions.

I may add that experiments exactly similar were soon afterwards made with peas, lentils, beans, and hemp seed. Except in the beans, the result so far corresponded, since a greater number of animalcula appeared in the infusions that had boiled most.

It is a fact established by the universal concurrence of philosophers that after water has come to the state of ebullition, it cannot acquire a greater degree of heat, however much the action of the fire may be augmented, provided it can evaporate. Therefore, when I say the seeds boiled longest acquired greatest heat, I mean it to be understood in time and not intensity, by supposing that the duration of boiling increased the intensity of heat the seeds would be exposed to.

Another experiment was undertaken to learn whether an increase of heat would obstruct the production of animalcula. The eleven species of seeds were slowly heated in a coffee roaster till they became pretty well roasted, and eleven infusions were formed of them with water previously boiled as usual. But this heat, so much more intense, neither prevented the origin of animalcula nor lessened the number. They were rare at first; but about the middle of October, that is, 20 days after making the infusions, the fluid was so full as absolutely to appear animated.

This constancy of their appearing, even here, excited my curiosity to augment the heat still more. The seeds were burnt and ground the same as we burn and grind coffee. Of the dust, which resembled soot, I made as many infusions as different kinds of seed; likewise, an infusion was made of the yolk of an egg, which by the thermometer had suffered 279° of heat.* What followed? Animalcula appeared in these infusions too, though a little more time elapsed before they became so numerous, because the weather was colder; and they uniformly inhabit infusions sooner or later according to the temperature of the atmosphere.

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*The author used Réaumur's thermometer in all his experiments. As Fahrenheit's is the only thermometer used in this country [Scotland and England], the degrees of heat are here reduced to his standard, 2.25° of Fahrenheit being equal to 1° of Réaumur. The fractional parts of the former are not given, because experiments can seldom be made within parts of a degree, or thermometers to agree exactly, and because the difference here, where there is any, never exceeds 0.25 of 1°. *Translator.*

Vegetable seeds were exposed to more severe trials: They were exposed to the greatest heat that can be produced by common fires, or fire augmented by artifice. Burning coals and the flame of the blow pipe were the two agents exercising their power on these seeds. In the first place, I kept them on an iron plate over burning coals until they were entirely consumed by the violence of the flame, and converted to a dry cinder, which was reduced to powder; and then I made as many infusions as there were seeds. A cinder was also made with the blow pipe, and this, besides excessive aridity, had acquired considerable hardness. I must acknowledge that I did not in the least expect to find animalcula in this new infusion. After viewing them again and again, hardly able to credit my eyes, I repeated the experiment twice. Some suspicion arose that the animalcula might come from the water used rather than the burnt seeds; therefore, on repeating the experiment, the same water used to make the infusions was put in other vessels. Both times, however, animalcula appeared in the fusions of burnt seeds, whereas not one was seen in the water alone.

These facts fully convinced me that vegetable seeds never fail to produce animalcula, though exposed to the highest degree of heat. Whence arises a direct conclusion that the *vegetative power* is nothing but the work of imagination; and if no animalcula appear in vessels hermetically sealed and kept an hour in boiling water, their absence must proceed from some other cause.

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II.

THEODOR SCHWANN

(1810 to 1882)

Theodor Schwann, one of the founders of modern histology, was educated at the Universities of Cologne, Bonn, and Würzburg (J. L. Schönlein's clinic), and earned his degree in medicine from the University of Berlin in 1834 with a dissertation on the respiration of the chick embryo. Subsequently, Johannes Müller, with whom he had been associated previously at Bonn, appointed him assistant in experimental physiology. In 1836, Schwann discovered pepsin during the course of studies on digestive processes, and in 1837 he demonstrated the relationship between organisms and fermentation and putrefaction. In fact, some consider Schwann the founder of the germ theory of fermentation since he demonstrated the real nature of the yeast cell.

In 1839, shortly after being appointed professor of anatomy at the University of Louvain, he published his famous *Microscopische Untersuchungen über die Übereinstimmung in der Struktur und dem Wachstum der Tiere und Pflanzen.* In 1847 he went to Liège as professor of physiology and comparative anatomy, and he remained in that post until his retirement in 1880. Schwann was born at Neuss, Rhenish Prussia, on December 7th, 1810, and he died in Liège on January 11th, 1882.

Schwann demonstrated in the paper presented here, that something in air, destroyed by heating, was responsible for fermentation and putrefaction, and thus he made a solid contribution to disproving the theory of spontaneous generation. He carried on the work begun by Spallanzani, on the one hand, and anticipated Pasteur's approach on the other, particularly in that he considered fermentation to be the result of a chemical decomposition caused by living yeast cells.

More interested in verifying the cellular origin and development of tissues, Schwann did not pursue the fermentation investigations which he had so brilliantly inaugurated, and which were to occupy many famous scientific personages for many years after 1836. He did, however, write a brief note concerning yeasts as fungi in his *Micro-*

National Library of Medicine

Theodor Schwann

scopische Untersuchungen. Schwann's paper which follows, is entitled "Vorläufige Mitteilung Betreffend Versuche über die Weingährung und Faulniss," and was published in *Annalen der Physik und Chemie,* XLI (1837), 184-193. It had been presented orally by his friend and teacher, Müller, on his behalf, February 1st, 1837.

PRELIMINARY COMMUNICATION CONCERNING INVESTIGATIONS ON ALCOHOLIC FERMENTATION AND PUTREFACTION

At the last meeting of natural science investigators in Jena, I presented work on *generatio aequivoca,* in which it was stated that if a sealed flask containing ordinary air and a meat infusion was heated to 80° R. [100° C.], no development of infusoria or putrefaction was noted after one month and, further, that the amount of animal matter in the flask was so minute that complete utilization of the oxygen within the flask was impossible. Later it was considered worthwhile to modify the experiment by allowing new air to enter and raising the flask to a higher temperature. This was accomplished in the following manner:

A flask containing a small piece of meat was filled one-third full with water and stoppered, and then two glass tubes were passed through the stopper. These tubes were led for a distance of about three inches through a fusible metal mixture which was continuously held near the boiling point of mercury. The end of one of these tubes was connected to a gasometer. The liquid in the flask was boiled vigorously, and the air was partially expelled through the tubes, at the same time being heated to the boiling point of water. After cooling, air was passed from the gasometer through the first tube; and after the air was renewed in the flask, it was removed via the second tube. The entering air traversed the tube in the metal bath and thus was heated to a high temperature. This study, of which more will be said later, showed that there was no development of infusoria or putrefaction after many weeks, and the liquid therein remained in the same condition as when it was first heated.[1]

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[1] There were some difficulties in adjusting the spirit lamp, but I finally developed the following method: A 3-ounce flask was filled one-quarter with water, and a bit of meat was added; the flask was then stoppered with a thick cork tightly bound with wire. Two glass tubes were led through the cork, and the first was bent downwards and the end placed in a small dish of mercury covered with oil. The second tube was bent in such a way that it came out of

Many other experiments are required in order to complete this work on *generatio aequivoca,* and these I shall consider elsewhere. I note here only that if one assumed the point of view of the opponents of *generatio aequivoca,* namely, that the spores of molds and infusoria are carried in the air, then it is these spores that are destroyed by heating the incoming air. Thus putrefaction may be explained as the development of spores which consume organic substances as food and thereby bring about the decomposition which constitutes putrefaction.[2] An observation that supports this view is that the same materials which are known poisons for infusoria and fungi, for example, arsenic or corrosive sublimate, are best for preventing putrefaction; and a substance toxic for infusoria, such as *Extractum Nucis vomicae spirituosum,* but not for fungi, allows the development of decomposition characteristic of fungi without hydrogen sulfide formation or other signs of infusorial activity.

I mention this point here because it leads to my studies on alcoholic fermentation. These were done in order to discover whether differences existed in processes concerned with atmospheric air, of which

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the cork horizontally and after one and a half inches bent downward, then it made a pair of narrow spirals inclined upward, and then horizontally, and finally ended in a point. The entire cork had been dipped several times in a heavy solution of gum boiled in turpentine-thinned linseed oil. The liquid in the flask was boiled vigorously so that steam issued from both tubes, and the mercury and oil were heated until no water or condensation remained. To prevent the development of infusoria in any water remaining between the oil and mercury, a small piece of sublimate was placed on the latter. During the boiling operation a spirit lamp was placed under the spirals of the second glass tube, and a chimney was provided to prevent the flame from wavering. The heat was so intense that the glass tube was softened. Water droplets which condensed in the cooler part of the tube thus were changed again to steam, and this prevented cracking. After heating the flask 15 minutes, it was allowed to cool and air entered it after first traversing the heated spirals. After complete cooling of the contents of the flask, the tip of the spiral was sealed by fusing it and the spirit lamp was removed. Now the flask contained only air that had been heated, and heated meat infusion. To renew the air in the flask periodically, the spiral again was heated to the point of softening, the tip end of the tube was broken off, and new air was slowly blown in, while the old air left via the mercury-oil bath. The end was again sealed. In this way I have kept a flask with meat infusion at 14° to 20° R. [17.5° to 25° C.]for six weeks without putrefaction, or development of infusoria or molds. After I opened the flask, the fluid putrefied after one day, as if the infusion had been merely boiled. Closure of the flask and glass tubes requires the greatest care.

[2] We shall speak here only of ordinary putrefaction, of that which follows shortly after death, or in cooked organic substances, rather than of the manifold processes that we might include under the designation of putrefaction, for example, mold formation, peat and mineral coal formation, etc.

much is known, in which no new plants or animals are generated, and for which heated air is of no consequence, and those under study here. Indeed I have already shown that a frog will thrive in heated air.

I made a study of alcoholic fermentation in the following manner. A solution of sucrose was mixed with beer yeast, and four flasks containing this were filled and sealed. The flasks were held in boiling water for ten minutes to heat the entire mixture to the temperature of the water. They were removed and inverted under mercury, and after cooling, air was allowed to enter all the flasks, in the amount of about one-third to one-quarter of their volume. In two of these flasks the air entered through glass tubes heated to redness, whereas in the other two the tubes were not heated. An analysis, using a platinum pellet, showed that the air in the heated flasks contained approximately 19.4 per cent oxygen. To the objection that any small difference found here would perhaps be reflected in some flasks containing heated air in still greater changes in oxygen content, I did the following: The flasks were sealed and kept at 10° to 14° R. [12.5° to 17.5° C.]. After four to six weeks a fermentation occurred in the two flasks to which unheated air had been added. The other two flasks after double this time are still unaltered.[3]

Oxygen is not the cause of fermentation or putrefaction, at least not the oxygen of the atmosphere; but that which motivates both these processes is something found in the air, a substance which is destroyed by heat.

One is led to believe that perhaps alcoholic fermentation is a transformation of sugar caused by the development of infusoria or some plant. Since *Extractum Nucis vomicae spirituosum* poisons infusoria but not fungi, and arsenic kills not only the former but most kinds of fungi, arsenic was used to ascertain whether my observation applied more correctly to infusoria or plants. It was found that a single drop of potassium arsenite will inhibit alcoholic fermentation, whereas the extract is ineffective. It is apparent that we must expect a plant to be involved.

A microscopic examination of beer yeast reveals the well-known

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[3] Later repetition of this experiment did not always yield the same result; sometimes fermentation did not occur in any one of the flasks (perhaps because they were all boiled too long); sometimes it occurred in flasks that contained only air that had been heated. These occurrences could be explained by the errors inherent in the experiment—by a variation in the surface of the mercury, even though this was heated to a high temperature, or by the entrance of some unheated organic matter in replacing the stoppers. Some methods are not applicable in studying putrefaction because of the long heating required. I would not have to mention these results, if once the existence of a plant were proved, for a plant would lead to putrefaction and mold development. The problem will be solved by using more exact methods.

spherules which constitute the ferment, and I saw at once that they clung together in rows. They are oval, mainly of a yellow-white color; some are single, and others appear in a series of two to eight together. One row is usually diagonally situated from a second row. Frequently one observes between two spherules a smaller spherule, which is the basis for a new row, and it is generally the smallest and drawn out lengthwise. In short, its over-all appearance is greatly similar to that of the reticulated fungi, and it is without doubt a plant.

Professor Meyen, who at my request was kind enough to examine this material, was entirely of the same opinion. He confirmed my observation that it was clearly more like a filamentous fungus than an alga because it lacked a green pigment.

Beer yeast is composed almost entirely of this fungus. No such a fungus is observed in freshly pressed grape juice, but if one incubates it at approximately 20° R. [25° C.], one discovers that at the end of 36 hours it contains similar plants. Under the microscope, these may be seen to grow; and after one-half to one hour an increase in volume of a small spherule, which is attached to a larger one, may be seen. Only after a lapse of an hour is gas evolved, because the initial carbonic acid remains dissolved. The formation of such plants continues during the course of the fermentation, and at the end a large quantity of yellow-white sediment may be seen in the bottom of the vessel. There are only small differences between these organisms and beer yeasts, among them the fact that most appear more oval and do not lie regularly in a line. Moreover, the number of spherules containing a second outgrowing spherule is far more numerous in fermented grape juice than in beer. These observations on the growth of the former leave no doubt concerning their identity as plants.[4]

The following points are established from this work:

1) A boiled organic substance, or a boiled, previously fermentable liquid, neither putrefies nor ferments if the air introduced into it has been heated.

2) In putrefaction and fermentation, and in general where new animals or plants are formed, there must be either unboiled organic matter or unheated air.[5]

3) The gas evolved from grape juice is the same evolved in fermentation, and is connected with a filamentous fungus, which might be

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[4] If meat or gelatin is added to a sugar solution and allowed to stand for some time, there are found therein similar plants, but fewer in number and deformed to some extent.

[5] It appears that unheated blood, removed from the vessels of living animals, does not putrefy if it is placed in a container in which there is air previously heated to the temperature of boiling water. This work ought be repeated several more times.

The laboratory digestion of protein is done in a manner that suffices to prevent putrefaction, but not stop digestion.

called a sugar fungus. During fermentation these plants grow, and their numbers increase.

4) The ferment is already present in formed plants, and fermentation occurs more quickly when formed plants are introduced into a sugar solution than when the plants must first produce it during growth.

5) Poisons effective against infusoria, but not lower plants *(Extractum Nucis vomicae spirituosum),* inhibit putrefaction phenomena characteristic of infusoria, but they do not inhibit fermentation, or the decomposition phenomena associated with fungi; poisons toxic to animals and plants (arsenic) inhibit putrefaction as well as fermentation.

The relationship between fermentation and the development of sugar fungi cannot be misinterpreted, for it is highly probable that sugar fungi activate the phenomenon of fermentation during their development. In addition to sugar, a nitrogen-containing body is necessary for fermentation; this is, of course, required for the continued life of any plant, and fungi are no exception. One may suppose the alcoholic fermentation to be a transformation of sugar mediated by the sugar fungus and a nitrogen-containing body, which is necessary for the growth and development of the fungus; and materials other than those within the plant are then combined to produce alcohol. This explanation is the most consistent with observations made on alcoholic fermentation. I will not commit myself concerning the final result of this investigation in this preliminary communication, and I refer the reader to other data on fermentation, as well as putrefaction and *generatio aequivoca,* in my forthcoming "Physiological Contributions."

The text of this paper is an unaltered copy of a paper which on February 1st of this year was read in my name to the local society of natural scientists by Professor Müller. Soon thereafter I obtained a copy of *L'Institut* of November 23d, 1836, in which I read that Charles Cagniard de la Tour made similar observations, unknown to me, on the fermentation of beer. He observed in beer mash, a half-hour after addition of yeast, isolated spherules, similar to yeasts. An hour later there were filaments of doubled spherules, which appeared to rise from the first one. Later, no simple spherules could be found; most were doubled and many consisted of three and four together. At the same time the numbers of spherules increased significantly, about sevenfold over the original number added. He concluded from further comparisons of spherules in mash and in fermented Johannisbeer and raisin juice, when no yeasts were added, that the spherules in the mash were *younger* than the other yeasts, and that the latter *during their activity in the mash sent out seeds that at once developed.* He actually twice observed the emanation of some fluid from the spherules. These are the chief points of Cagniard de la Tour's communication. Briefly, this esteemed French investigator has published a work on the fermentation of beer which I have not yet received and of

whose content I am aware only from nonscientific accounts. I cannot judge how far we are in agreement on the main points and details.

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III.

LOUIS PASTEUR

(1822 to 1895)

Louis Pasteur was born in Dôle, Franche-Comté, France, on December 27th, 1822, and he died on September 28th, 1895, at Villeneuve l'Etang, near Paris. He was sent to schools at Arbois and in 1838 went to Paris to study at the Institution Barbet. However, because of ill health, he returned to the Franche-Comté, and from 1839 to 1842 attended the Collège Royal de Besançon. In 1843 he was admitted to the Ecole Normale Supérieure, and there he received his first incentive to study chemistry from hearing the lectures of J. B. A. Dumas at the Sorbonne. In 1847 he obtained the doctorate and began his well-known work on isomerism of tartaric acid enantiomorphic forms. In 1853 he received the Prix de la Société de Pharmacie de Paris, and in the same year he was made a member of the Légion d'Honneur. The Royal Society of London awarded him the Rumford medal (1857) for his brilliant work on the relation of crystal structure to optical activity.

Pasteur taught at the Universities of Dijon and Strasbourg from 1848 to 1854, and in the latter year was appointed dean and professor of chemistry of the newly organized Faculté des Sciences at Lille.

In the following year, he turned his attention to the study of fermentations and thus initiated the classical work which was to destroy the notion of spontaneous generation as then conceived, and ultimately lead to the uncovering of the relationship of specific microorganisms to disease. He made brilliant studies not only of the diseases of beer and wine (1866 to 1876) but also of silkworm diseases (1865 to 1869), anthrax (1877 to 1881), gangrene, septicemia, and childbirth fever (1878), rabies (1880 to 1887), fowl cholera (1880), and swine erysipelas (1882).

From 1857 on, he spent most of his time in Paris as director of the Ecole Normale Supérieure (1857 to 1863), professor of chemistry at the Sorbonne (1867 to 1889), and head of the Institut Pasteur, which was founded in 1888.

Although Pasteur became partially paralyzed in 1868, he would not let this interfere with his great work. This fierce determination is characteristic of him, for throughout his life he refused to acknowledge defeat either before overwhelming personal circumstances or in scientific controversies.

When F. A. Pouchet (1859) published his ideas on heterogenesis, it became imperative for Pasteur to prove this, and thus he was forced into the work on spontaneous generation.

In Pasteur's words in the first chapter of his classic "Mémoire sur les Corpuscles Organisés Qui Existent dans l'Atmosphère: Examen de la Doctrine de Générations Spontanées" (1861): ". . . all fermentations properly so-called—lactic, butyric, the fermentations of tartaric acid, malic acid, and urea, of sugar leading to mannite production—were always associated with the presence and multiplication of living organisms. . . . Accordingly albuminous materials are never ferments, but nutrients for ferments. True ferments are living organisms. . . . Then, I decided, one of two possibilities are true. Since true ferments are living organisms, if they are produced by contact of albuminous matter with oxygen as such, then they are spontaneously generated. If living ferments are not spontaneously generated, then it is not oxygen as such that causes their appearance, but that oxygen is a stimulent to the organism already carried in it, or existing in the albuminous or fermentable material. At this point . . . I was obliged to decide on the question of spontaneous generation."

Pasteur believed that the presence of optically active amyl alcohol as a product of the alcoholic fermentation of sugar indicated the mediation of a living organism. Although he worked extensively on the alcoholic fermentation during 1856 and 1857, his first publications on the subject dealt with the lactic fermentation. It is curious that he considered the organism responsible for this transformation to be a yeast, his so-called "lactic yeast." Pasteur was successful in demonstrating that microorganisms were the cause, rather than the consequence of, fermentation, and he suggested that each kind of fermentation is due to the presence of a specific organism. The ramifications of this work into areas now designated as "medical bacteriology" and "bacterial physiology" are readily seen in this work.

The following paper appeared as an abstract entitled "Mémoire sur la Fermentation Appelée Lactique." It was published in the *Comptes Rendus de l'Académie des Sciences,* Paris, XLV (1857), 913-916. The following year a much longer paper on the same subject was published, and this is cited at the end of the chapter.

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Louis Pasteur. Painting by Albert Edelfelt.

REPORT ON LACTIC FERMENTATION

I have been conducting work on fermentation as a consequence of my investigations on the properties of amyl alcohols and the particulars of the very remarkable crystallography of their derivatives. At last I have the honor of presenting to the Academy of Sciences observations which afford an unexpected connection between the phenomena of fermentation and the dysymmetric character of the molecules of natural organic substances. . . .

The material conditions for the preparation and production of lactic acid are well known to chemists. It is sufficient to add to sugar water and chalk, which latter keeps the liquid neutral, a nitrogenous substance such as casein, gluten, or animal membrane in order to transform the sugar into lactic acid. Explanations of this phenomenon are very obscure and do not take into account all the facts on the mode of action of the added nitrogenous material. The weight of this material is not changed perceptibly, and it is not putrefied. It is changed, nevertheless, and it is continuously in a state of alteration, although it would be difficult to say of what this consists.

In scrupulous investigations it has been impossible to discover in this phenomenon the necessary development of organized beings. Observers who accept this have considered that organized beings are accidental or harmful to the reaction.

The facts therefore appear very favorable to the ideas of J. Liebig.[1] To him a ferment is "an extremely alterable substance that decomposes and excites fermentation, and as a consequence of its alteration, communicates a disturbance to the molecular group of the fermentable material." This, according to Liebig, is the primary cause of all fermentations and the origin of most contagious diseases. His view continually gains more credit. On this point one may consult the paper of E. Fremy and A. F. Boutron on lactic fermentation, the fine work C. Gerhardt left upon his death, and finally, the recent report of M. Berthelot on alcoholic fermentation. These works reject the idea that living beings can be a cause of the phenomena they studied. I have been led to an entirely different point of view.

In the first part of this work I propose to establish that just as an alcoholic ferment exists, brewer's yeast, which is found wherever sugar is decomposed to alcohol and carbonic acid, so also there is a special ferment, a lactic yeast, always present when sugar is changed to lactic acid; and if any nitrogenous matter is able to transform sugar

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[1]See results of recent historical researches by E. Chevreul presented in the *Journal des Savants*, in which G. E. Stahl is said to have stated an idea analogous to that of J. Liebig on the causes of alcoholic fermentation.

into this acid, it is because it serves as a food suitable for the development of the ferment.

In a careful examination of an ordinary lactic fermentation, one may find on top of the deposit of chalk and nitrogenous matter, spots of a gray substance which form a layer on the surface of the deposit. It is impossible to identify it under the microscope as casein, disaggregated gluten, or some other material, so that there is nothing to indicate whether it is a distinguishable component of the original mixture or whether it was formed during the fermentation. It is this material which nevertheless plays the principal role. I am going to describe, first of all, how to isolate and prepare it in a pure state.

I extract the soluble part of brewer's yeast by placing it for some time in fifteen to twenty times its weight of boiling water, and then filtering the liquid with care. One then dissolves 50 to 100 gm. of sugar per liter, adjusts with chalk, and adds a trace of the gray material I mentioned from an ordinary lactic fermentation. The next day, there is a strong and regular fermentation. The liquid, originally limpid, becomes turbid; the chalk dissolves little by little, and at the same time the deposit is formed and grows continuously and in proportion as the chalk is dissolved. Meantime, one observes all of the characteristics of the lactic fermentation. In this experiment, one may substitute for yeast extract any nitrogenous material, fresh or altered, as the case may be. I maintain that this characteristic gray matter is produced correlative with the phenomenon which we call lactic fermentation. Viewed whole, it looks exactly like ordinary pressed or drained yeast. Microscopically, it is formed of little globules or very short, segmented filaments, isolated or in clusters. These globules, much smaller than those of brewer's yeast, are rapidly agitated by Brownian movement. By decanting a large amount of liquid, and adding pure sugar to it, acid formation begins at once, but at a slower rate, since the acidity somewhat inhibits the activity of the ferments on sugar. If one adds chalk, which maintains the neutrality of the medium, the transformation of sugar is accelerated; and even if one uses very little material, in less than one hour gas is evolved and the calcium salts of lactic and butyric acid are formed. Very little ferment is required to change a large amount of sugar. This fermentation proceeds better in the absence of air, and in addition there is brought about the growth of the infusoria. . . .

Lactic fermentation is similar in nature to the ordinary alcoholic fermentation, and it is a correlative act of production of nitrogenous matter which is like an organized mycoderm body or brewer's yeast. The difficulties of this subject are but half resolved, and there are extreme complications. Lactic acid is certainly the principal product of the fermentation for which it is named. It is far from being the only product. One obtains butyric acid, alcohol, mannite, and slimy material. The proportions of these products are subject to capricious

variation.* The occurrence of mannite is puzzling; not only are the proportions formed subject to great variation, but, in addition, as Berthelot has established, if one substitutes mannite for sugar in the lactic fermentation, all other conditions remaining the same, fermentation of mannite yields alcohol and lactic and butyric acids. How can the formation of mannite in the lactic fermentation be explained, and how can one interpret the fact that both in its destruction and formation lactic acid is produced?

We need careful studies to obtain the true facts about the chemical properties of this new yeast. I stated that if the liquid was decanted and pure sugar added, the liquid was progressively acidified. Under these conditions one finds the transformation of sugar more and more difficult as the acidity increases. If one analyzes the liquid, this being done with success only after neutralizing the acids with chalk and removing the excess sugar by fermentation with brewer's yeast, one always finds variable proportions of mannite and viscous material in the residual liquid. Hence, the lactic yeast prepared in the presence of sugar transforms it into diverse products, among which there is always mannite, provided the liquid promptly becomes acid; for on repeating the experiment with the precaution of adding chalk so that the medium is constantly neutral, neither viscous material nor mannite is formed, or rather does not persist. . . .

I recalled previously that Berthelot showed that mannite was fermented when it was substituted for sugar in the lactic fermentation. One is easily convinced that in mannite fermentation it is the lactic yeast which produces the phenomenon. If one takes a pure solution of mannite to which is added powdered chalk, and fresh, washed lactic yeast, gas production and the chemical transformation of mannite are observed at the end of one hour. Carbonic acid and hydrogen are evolved, and the liquid contains alcohol and lactic and butyric acids.

Concerning the butyric acid, these experiments prove that the lactic yeast acts directly on calcium lactate and yields calcium carbonate and calcium butyrate. The action is first directed to the sugar, and as long as some is present, the yeast continually forms lactic acid.

In this short communication, I have had the honor of presenting to the Academy of Sciences the application of general ideas and new methods of experimentation in work on these fermentations.

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IV.

FERDINAND JULIUS COHN

(1828 to 1898)

Ferdinand Julius Cohn was born in Breslau, Germany, on January 24th, 1828, and was educated at the Universities of Breslau and Berlin. At the latter institution he studied under Eilhardt Mitscherlich, Johannes Müller, and C. G. Ehrenberg. Cohn's interest in microscopic studies was stimulated by Ehrenberg, and when he returned to Breslau as professor of botany (1859), he applied himself to a study of the life histories of a variety of microorganisms. His descriptions of the life cycles of several genera of algae are still valid.

Cohn soon extended his investigations to studies of fungi and bacteria of air and water, and today he is recognized as one of the pioneers of bacteriology. He founded the *Beiträge zur Biologie der Pflanzen;* and by his papers on bacterial classification, "Untersuchungen über Bakterien" (1872 to 1875), he helped unravel some of the confused ideas concerning the taxonomy of bacteria.

Cohn's work was not confined exclusively to bacteria, and his studies included a variety of subjects among which were torsion in trees, physiology of seeds, proteid crystals of potatoes, rotatoria, and luminous worms. He is also known to microbiologists for his work on the organism producing a blood-red pigment *Monas prodigiosa (Serratia marcescens)* (1850), microorganisms in drinking water (1853), an investigation of some significance since J. Snow (1849) and W. Budd (1849) had shown the significance of drinking water in the spread of cholera, and of *Crenothrix,* a genus of iron bacteria found in public water supplies (1870). In 1876 he aided Robert Koch in the preparation of his classic paper on anthrax, justly celebrated as the first positive experimental demonstration of a bacterium as a disease agent. In the same year he discovered the existence of spores in *Bacillus subtilis,* and his observations on their remarkable resistance to heat had great bearing on the current arguments concerning spontaneous generation. Cohn was a foreign member of the Royal Society of London and the Linnean Society, and he received the Gold Medal

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Ferdinand Julius Cohn

award of the latter in 1895. He died in Breslau on June 25th, 1898.

The essay presented here is a summary of the state of knowledge concerning the biology of bacteria in the early 1870's. When it is remembered that much of the great work of Koch and Pasteur was yet to come, it is indeed remarkable for its breadth of understanding; it is of particular interest that Cohn considers bacteria from a biological, rather than from a medical, point of view. Cohn was aware of the problem of whether bacteria could validly be classified in genera and species. Most of his characteristics were based on morphology, but he recognized the insufficiency of this, because of their small size and the paucity of features that could be employed. He also recognized that morphologically similar entities differed from one another physiologically, and he designed his groupings as "form genera" and "form species," leaving the problem of their final disposition to the time when more information became available. At any rate Cohn believed that bacteria were characterized by a constancy of form, not subject to capricious variations, a doctrine later crystallized into the so-called "Cohn-Koch dogma."

The first edition of *Über Bakterien, die Kleinsten Lebenden Wesen* was published in Berlin in 1872. The work was translated by a medical student at the University of Pennsylvania, Charles S. Dolley, in 1881, and published in Rochester, New York. It was reprinted in the *Bulletin of the History of Medicine,* VII (1939), 49-92, with an introduction by M. Leikind. The work presented here has been slightly adapted.

BACTERIA, THE SMALLEST OF LIVING ORGANISMS

In the year 1875, science celebrated the second centennial of the discovery of a new world by Anton van Leeuwenhoek, who though he had not received a classical education, was gifted with a strong desire for investigation; the seventeenth century, the age of the greatest discoveries in natural science, animated him as well as many other men with gifted minds.

Leeuwenhoek early in his youth left the merchant's shop in Amsterdam, which he had entered as an apprentice, and contented himself with the position of janitor of the court room in his native Delft, where he served thirty-nine years; but his spare time and his great mechanical talent he applied to the manufacture of magnifying glasses, with which he, commencing as an amateur, observed gnat wings, bee stings, butterfly scales, and mosses.

But with the new perfections of his microscope, and his clear and persevering gift of observation, there were soon revealed to him "deep secrets of nature," which he communicated to the Royal Society of Sciences in London in enthusiastic letters. In April, 1675, Leeuwenhoek placed beneath his microscope a glass tube full of standing rain water, and with astonished surprise he noticed in the water wonderful forms—little bells which expanded and contracted themselves, and little globules which shot quickly here and there. At first he thought he saw the living atoms which, according to the philosophy of Democritus, composed all bodies, and from whose whirling motion his contemporary, Descartes, believed the world built itself anew. But Leeuwenhoek soon became satisfied that these were animalcules, invisible to the naked eye, which in varied forms animated the drop of water. Other animal and plant forms were found later in great abundance, in infusions of pepper, hay, and other animal and vegetal materials, and for that reason were named *Infusoria*. Exactly a century after Leeuwenhoek, O. F. Müller, an investigator in Denmark, having devoted twelve years of his life to the observation of these smallest of creatures, found, named, and described about 380 different forms in the fresh and salt waters of Copenhagen.

In the next century there was a rapid increase in the number of observers who, with still more perfect instruments, sought to penetrate the unseen world. Aside from the numerous animal race, a very peculiar microscopic flora was discovered, the appearance and development of which differed entirely from those of visible plants. If Leeuwenhoek was the Columbus of this new world, we may designate C. G. Ehrenberg the Humboldt of the same; for from the year 1829 up to the present day Ehrenberg has, with indefatigable diligence, thoroughly investigated these hidden provinces; not only has he described, delineated, and classified the microscopical beings more thoroughly and more truly than his predecessors, but also he has unveiled the unsuspected importance that was due to creatures of the invisible world, in the entire natural scheme, not only in the present but in former geological ages.

Everyone knows in how many different sizes the life of the visible world embodies itself. The mites are among the smallest creatures visible to the naked eye. They are found in numberless swarms in cheese and in fruits rich in sugar. Their size compares to that of man about as a sparrow to the Strasbourg Cathedral. A similar comparison may be made between the giant fir tree and the moss which grows on its bark. Of the little animalcules that Leeuwenhoek discovered, he stated that their size compared with that of the mite as the bee with the horse. The more the microscope has been improved, and its magnifying power increased, the smaller have been the beings that have become accessible to keen observation, since among the animals and plants of the unseen world, a difference in size is found, similar to that between the herring and the whale.

But the smaller the organism, the simpler appears to be its form, the more imperfect its life energy, and the lower its place in the rank of created beings. Among the animals of the microscopic world, we find exceedingly few that possess the fullness of organs of an insect, a crab, or even a worm: The true infusoria stand on the lowest step of the animal kingdom. Even so, among microscopic plants we find not one that reaches the developed form of the blooming plant, or belongs to even the lowest class of the ferns; only the lowest plant forms, which we usually designate as algae and fungi, form the forests and meadows of the invisible world.

But the more the inner formation of microscopic organisms is simplified, the fewer appear to be the characteristics which so easily separate plants and animals in the visible world. The infusoria are wanting in muscle and nerve, while vessels and breathing organs are very imperfectly developed. On the other hand, microscopic plants show independent movement, and even organs of movement, such as we are accustomed to find only in animals. In the very lowest organisms, animals and plants appear to run into each other, and the naturalist is in doubt to which of the two kingdoms he shall assign the subject of his investigations.

But the smallest, and at the same time the simplest and lowest of all living forms, we call *Bacteria*.* They form the boundary line of life; beyond them life does not exist, at least not within the powers of our present microscopes; and these are not poor. The strongest of our magnifying lenses, the immersion system of Hartnack, gives a magnifying power of from 3,000 to 4,000 diameters; and could we view a man under such a lens, he would appear as large as Mont Blanc, or even Chimborazo. But even under this colossal amplification, the smallest bacteria do not appear larger than the points and commas of good print. Of their internal parts, little or nothing is to be distinguished, and even their existence would for the most part remain hidden, did they not live in such gregarious masses.† These smallest bacteria may be compared with man about as a grain of sand with Mont Blanc.

If it is important on their own account to learn to know these smallest and at the same time simplest of organisms, then will our interest be increased through the knowledge that these little forms are of the very greatest moment; since they, with invisible yet irresistible

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*From **βακτέριον** a little staff or rod. *Translator.*

†"So long as the makers of microscopes do not place at our disposal much higher powers and, as far as possible, without immersion, we shall find ourselves, in the domain of the bacteria, in the situation of a traveler who wanders in an unknown country at the hour of twilight, at the moment when the light of day no longer suffices to enable him clearly to distinguish objects, and when he is conscious that, notwithstanding all his precautions, he is liable to lose his way" (Cohn). *Translator.*

power, govern the most important processes of animate and inanimate nature, and even seize on the being of man secretly, but at the same time fatally.

The forms of the bacteria resemble sometimes balls or eggs, sometimes shorter or longer rods or fibers, and sometimes corkscrews or screws. The bodies consist of an almost colorless albuminous substance, in which numerous shining, fatty granules are embedded,* and which is inclosed in a thin membrane (cellulose), insoluble in caustic potash. According to their form, we can distinguish ball, rod, fiber, and screw bacteria.

They are divided by scientists into genera and species. The author of this paper has in his new work recognized six genera, as shown in Figure 1:

1. *Micrococcus,*	ball or egg-shaped	Nos. 1 to 7
2. *Bacterium,*	short, rod-like	Nos. 8 to 12
3. *Bacillus,*	straight, fiber-like	Nos. 14, 15
4. *Vibrio,*	wavy, curl-like	Nos. 16 to 18
5. *Spirillum,*	short, screw-like	Nos. 19 to 21
6. *Spirochaete,*	long, flexible, spiral	Nos. 22

Nearly all bacteria are capable of two different modes of life, one of motion and another of rest. In certain conditions they are excessively mobile, and when they swarm in a drop of water, moving amongst each other in all directions, they present an attractive spectacle, similar to that of a swarm of gnats or an ant hill. The bacteria swim rapidly forward, then, without turning about, retreat; or they may even describe circular lines. At one time they advance with a rocket-like spring, at another they turn upon themselves like a top; or they remain motionless for a long time, in order as quick as lightning to be up and away. The longer, fiber-like bacteria bend their bodies in swimming, sometimes sluggishly, sometimes with address and agility, as if they were troubled to find their way through some impediment, like a fish that seeks its way among aquatic plants. Sometimes the little fiber stands still a long time, as though it would rest; then suddenly it begins to tremble, and swims back, in order soon to steer forward again. All these movements are accompanied by a rapid turning on the axis, like a screw moving in the screw nut; this becomes especially apparent if the little rods are bent, for they appear to be giddily whirling around. When the wavy-formed vibrios and the screw-formed spirilla turn themselves quickly on their axes,

*Some of these granules have recently been found to consist of crystalline sulfur (Cramer, F. Cohn). They have been observed in *Monas Okenii* [*Chromatium okenii*], *Bacterium sulphuratum,* and in the different species of *Beggiatoa,* which latter are found most abundantly in thermal sulfur waters, where they play a great *role* in the elimination of sulfur and the disengagement of sulfurated hydrogen (Magnin). *Translator.*

they give rise to a curious delusion, as if they possessed an eel-like motion, although they are in reality perfectly rigid. They often, meteor-like, dart here and there through the water, so that the observer is scarcely conscious of their presence, or they roll rapidly through the field of vision; sometimes they fasten themselves by one end and move the other end in a circle, exactly like a sling around its cord; soon after, one sees them winding slowly through the water.

Nearly all the older observers regarded bacteria as animals, since their movements were considered to be voluntary.* Certainly it is inner life energy which causes the movements of the bacteria; and the power of movement is even more enigmatical, since no organs of motion are visible,† yet no doubt the appearance of volition is only a delusion. No mind energies, as they lie in our conception of volition, and which in fact govern the least of the actions of higher animals, are in play in the bacteria.

Exactly similar movements, as has been already remarked, are noticed in many microscopic plants, either continuing, as in the diatom and oscillaria, or transient during propagation, as in the zoospores or little seed bodies of the algae and fungi.

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*Ehrenberg was the first to maintain that the motion of bacteria depended upon the presence of vibratile cilia (observed by him in *Spirillum volutans*); "but although the cilia, denied at first by most microscopists, have been seen since in nearly all the bacteria, recent researches permit us to say that cilia exist without doubt in all the true bacteria; the botanists who have best studied them, E. Warming, for example, recognize that it is scarcely possible that these organs are the cause of their movements, for 'one meets some examples in which the body remains motionless while the cilia are in violent agitation, and others in which the body moves, while the cilia remain inert, or dragging behind'" (Magnin). *Translator.*

†Bacteria were regarded as animals up to the time of F. Dujardin (1841), a kingdom—the *Protista*—midway between the animal and vegetable, the term being created by E. Haeckel for their especial benefit. C. Davaine (1859) was, however, among the first to show clearly their alliance with the algae. Cohn holds them to belong to the algae, although, from their want of chlorophyll, approaching the fungi. Magnin says: "If there are still some differences of opinion among naturalists as to the place of the bacteria among the cryptogams, there is but one opinion as to their vegetable nature. H. Sachs, however, solves the question by uniting the algae and fungi in a single group, the *Thallophytes*, in which he establishes two series exactly parallel—one comprising the forms with chlorophyll, the other the forms which are deprived of it."

To quote Magnin again: "The bacteria, then, resemble green plants, in that they assimilate nitrogen contained in their cells by taking it from ammonia compounds, which animals cannot do. They differ from green plants, in that they cannot draw their carbon from carbonic acid, and only assimilate organic substances containing carbon, above all the hydrates of carbon and their derivatives; and in this respect they resemble animals." *Translator.*

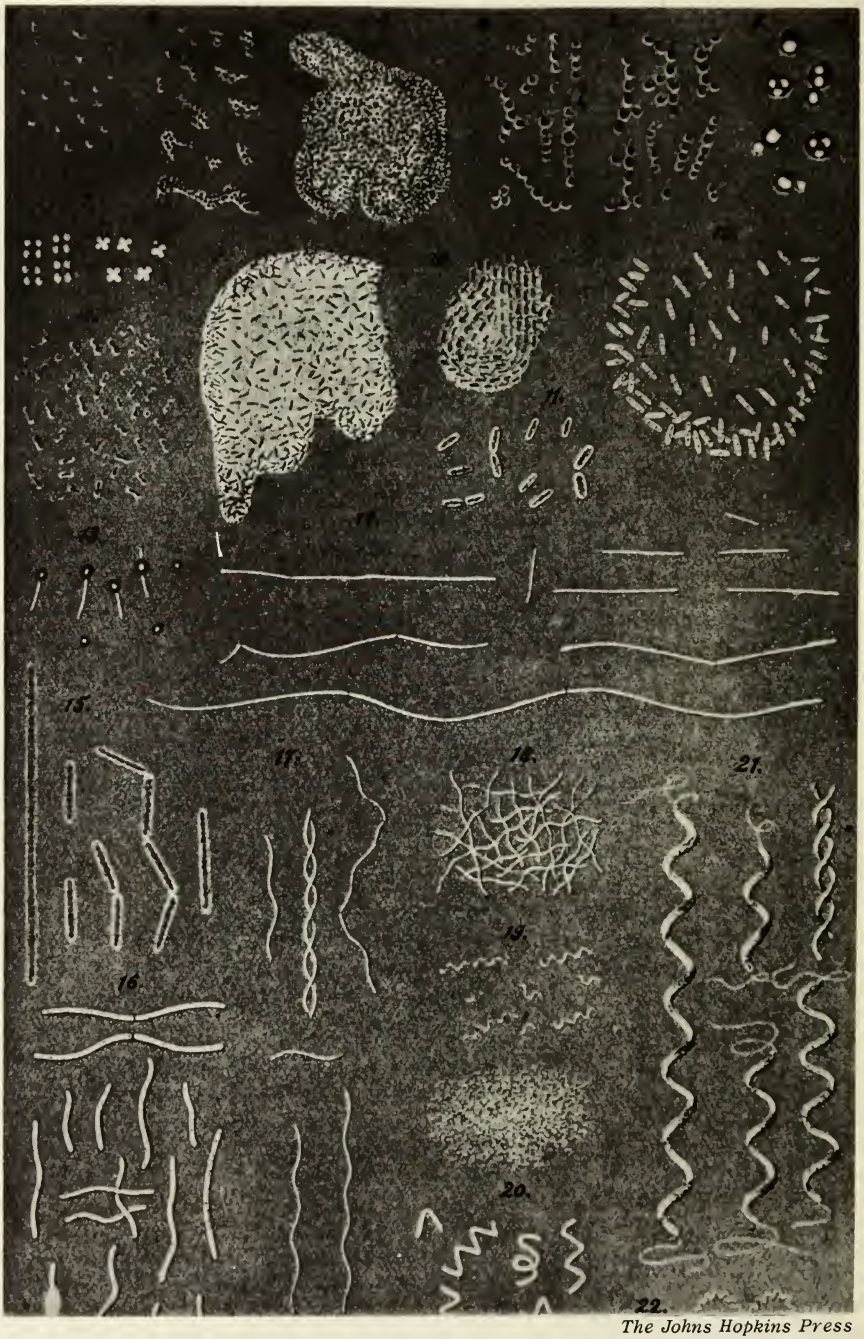

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Figure 1. Cohn's first plate illustrating bacteria. Courtesy M. Leikind.

The collective development of the bacteria makes it in the highest degree probable that they belong to the vegetable kingdom, and in the nearest relation to the *Oscillariae*. Bacteria also change from a condition of movement to one of rest, when they cannot be entirely distinguished from common plant cells. They swarm only where there is favorable temperature, plenty of nourishment, and available oxygen;

DESCRIPTION OF BACTERIA IN FIGURE 1

1. *Micrococcus prodigiosus* (*Monas prodigiosa,* Ehr.). Spherical bacteria of the red pigment, aggregated in pairs and in fours; the other pigment bacteria are not distinguishable with the microscope from this one.

2. *Micrococcus vaccinae.* Spherical bacteria, from pock-lymph in a state of growth, aggregated in short four to eight-jointed straight or bent chains, and forming also irregular cell-mass.

3. Zooglea—form of micrococcus, pellicles or mucous strata characterized by granule-like closely set spherules.

4. Rosary-chain (*Torula*-form) of *Micrococcus ureae,* from the urine.

5. Rosary-chain and yeast-like cell-masses from the white deposit of a solution of sugar of milk which had become sour.

6. *Saccharomyces glutinis* (*Cryptococcus glutinis,* Fersen.), a pullulating yeast which forms beautiful rose-colored patches on cooked potatoes.

7. Sarcina spec. . . . from the blood of a healthy man. . . . from the surface of a hen's egg grown over with *Micrococcus luteus,* forming yellow patches.

8. *Bacterium termo,* free motile form.

9. Zooglea form of *Bacterium termo.*

10. Bacterium-pellicle, formed by rod-shaped bacteria arranged one against the other in a linear fashion, from the surface of sour beer.

11. *Bacterium lineola,* free motile form.

12. Zooglea form of *B. lineola.*

13. Motile filamentous bacteria, with a spherical or elliptical, highly refringent "head," perhaps developed from gonidia.

14. *Bacillus subtilis,* short cylinders and longer, very flexible motile filaments, some of which are in process of division.

15. *Bacillus ulna,* single segments and longer threads, some breaking up into segments.

16. *Vibrio rugula,* single, or in process of division.

17. *Vibrio serpens,* longer or shorter threads, some dividing into bits, at . . . two threads entwined.

18. "Swarm" of *V. serpens,* the threads felted.

19. *Spirillum tenue,* single and felted into "swarms."

20. *Spirillum undula.*

21. *Spirillum volutans* . . . two spirals twisted around one another.

22. *Spirochaete plicatilis.*

All the figures were drawn by Dr. Ferdinand Cohn with the immersion lens No. IX. of Hartnack Ocular III, representing a magnifying power of about 650 diameters.

under unfavorable conditions they are motionless. Certain kinds of spherical bacteria appear never to move.*

Like all living organisms, bacteria are capable of propagating themselves. This propagation depends on bipartition ("fission"). The bacterium grows till it has reached perhaps double its original size, then constricts itself in the middle like a figure eight, and breaks into two new individuals; each of these in a short time divides again, and on account of the rapidity of this process we usually find them multiplying, either constricted in the middle or hanging together in pairs. The warmer the air, the faster proceeds the division and the stronger the multiplication; in a lower temperature it becomes slower, and it ceases entirely in the neighborhood of the freezing point.

It well repays the trouble to make a computation of the incredible masses to which these smallest of all organisms are capable of multiplying themselves. We know that bacteria divide themselves in the space of an hour into two parts, then again after another hour into four, after three hours into eight, etc. After 24 hours the number exceeds 16.5 million (16,777,220); at the end of two days this bacterium will have multiplied to the incredible number of 281.5 billion; at the end of three days it will have increased to 48 trillion; and after a week the number can only be expressed by figures of fifty-one places. In order to make this number comprehensible, we shall reckon the mass and weight which may result from the multiplication of a single bacterium. A single individual of the most common species of rod bacteria *(Bacterium termo)* has the appearance of a short cylinder 1/1,000 mm. in diameter, and perhaps 1/500 mm. in length. Let us now think of a cube, the side measuring a millimeter (a cubic millimeter); 633 million rod bacteria will completely fill this cavity without leaving any empty space. The fortieth part of a cubic millimeter would perhaps contain the bacteria that proceed from one single little rod in 24 hours; but at the end of the following day the bacteria would fill a space equal to 442,570 such cubes, or, what is the same, perhaps, half a liter, or 445 cc. Take the space which is occupied by the seas of this world, about two-thirds of the terrestrial surface, say with a mean depth of a mile, the collective contents of which would be 929 million cubic miles; by continual progression of multiplication the bacteria which spring from one germ would in less than five days fill the whole world's seas completely full; the number can only be expressed by figures of thirty-seven places.

Still more surprising are the proportional weights. If we call the specific weight of one bacterium equal to that of water, which cannot be far from the truth, it appears from the above-mentioned measure that a single little rod will weigh 0.000,000,001,571 mg. or that 636 billion bacteria would weigh 1 gm., or 636,000 billion a kg., after 24

Micrococcus, Cohn. *Bacillus anthracis*, Cohn. *Bacteridie charbonneuse* [*Bacillus anthracis*], Davaine. *Translator.*

hours the weight of the bacteria amounts to about 1/40 mg.; after 48 hours, nearly 1 lb. (442 gm.); after three days it approximates a weight of nearly 7.5 million kg., or 148,356 hundredweight.

We do not consider such computations idle play; they alone can make the immense work executed by the bacteria comprehensible to us. They also depend only on such hypotheses as nature herself presents to us; should, for example, the continuance of the process of fission be in truth somewhat longer than that stated by us, the numbers would agree a few hours or days later. Certainly, if in limited space this quantity is at no time reached, it perhaps does not signify that the power of multiplication in the bacteria falls below the calculation; rather the multiplication depends altogether on the amount of nutrition. It is self-evident that bacteria do not generate the material which forms their bodies, but take it in from without as food; and therefore no more can be formed than there is food provided for. It follows that other animals and plants have access to the same food, and they on their side strive for existence. The fierce combat for life, according to the old concept of the extermination of the weaker, holds the increase of the bacteria, as of all other beings, in limitation; and it is only where they hold the upper hand that they are able to keep off the rivals which at the same time are their deadly enemies.

A compressed yeast factory gives a plain example of the colossal proportions in which these little microscopic organisms can increase if abundance of nourishment is given them and they are carefully protected from the opposition of other beings. The yeast fungus exceeds the rod bacteria in mass and weight probably 160-fold.[1] The weight of a yeast cell is also about 0.000,000,25 mg. or 40 million yeast cells weigh 1 kg. If they are in great vats filled with suitable food and are allowed to remain undisturbed, inside of 24 hours over one hundredweight of yeast is generated. Probably more than 50 billion cells form such a mass in the course of one day from one single grain.

We as yet know of no other mode of increase in the bacteria beside bipartition. Generation of eggs and spores, as is found in all other animals and plants, has not yet been observed in these simplest forms.[2] After division, the halves of the bacteria separate and either

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[1] I accept a yeast cell as a spherule whose diameter is 0.008 mm. [8μ], and whose content is 0.000,000, 25 cc. In the compressed yeast factory of Giesmannsdorf, near Neisse, 100 cc. of yeast consists of 75 per cent water and 25 per cent yeast.

[2] Since writing the above, the author has satisfied himself not only of the existence of spores but of veritable sporangia, and has by experiment found that the spores of *Bacillus* will for several days withstand a temperature of 80° C. (176° F.), still retaining the power of germination, while, along with Frisch, he has proved that gradual freezing does not destroy them, development having occurred after exposure to a temperature of -87° C. (-123° F.).

wander about as independent organisms, or remain connected chain-like and form longer or shorter threads; in other cases whole generations remain congregated in colonies, united in nests or balls, or collected together in heaps, which appear to the naked eye as colorless or perhaps colored gelatinous or slimy masses,* as little white flakes or threads swimming in the water or settling as flakes to the bottom of the fluid.

Bacteria belong to the most widespread of organisms; we may say they are omnipresent; they never fail either in air or water; they attach themselves to the surface of all firm bodies, but they develop in masses only where decomposition, corruption, fermentation, or putrefaction is present. If we place a piece of flesh, a pea, or other animal or vegetable material in water, it will become, earlier or later, thick, and then milky. The water loses its transparency, because the bacteria completely fill the water; at the same time the putrefaction increases, under the development of different and, for the most part, bad smelling chemical combinations. After a time the thickness disappears, and the water becomes clear and odorless, the organic material is consumed by the bacteria; these now cease to divide themselves further, and heap themselves on the bottom without motion as white sediment. If a new supply of nutritive material be added to the fluid, putrefaction and the multiplication of bacteria which are not dead, but in a state of temporary repose, are seen to begin anew.

Bacteria also multiply without water in moist air wherever they can find decaying matter. They cover cheese, cooked potatoes, and other foods in damp closets with a slimy, colorless, or colored coating, which even with the naked eye is easily distinguished from the snow-white, web-like mold fungus sprinkled with blue-white spore

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He states that "reproduction by means of spores is only made under the influence of free access of air." Bacteria spores, besides their ability of resisting vicissitudes of temperature, will withstand complete desiccation; and Magnin says: "These spores are the point of departure of epidemic foci, and their extreme lightness explains how readily they are disseminated by the wind. It has been shown from the experiments of Cohn and P. Miquel that the atmosphere contains very few adult bacteria; while Cohn again proves that these germs are of so small a diameter that they pass through all filters."

*Cohn explains the origin of the gelatinous substance in which the bacteria are included as being produced by a thickening or jellification of the cell membrane; but a more plausible view is that it is produced by a secretion from their protoplasm. It is commonly the spherical bacteria (*Micrococcus*) and the microbacteria (*Bacterium*) which are found in this state. The bacteria are of course motionless in the zooglea on account of the intermediary glairy substance" (Magnin).

The filliform bacteria and the spirilla, according to Cohn, are never found in gelatinous masses, but may be found in active swarms. *Translator.*

powder. A great part of the whitish slime that collects on the teeth is composed of bacteria *(Vibrio regula [Spirillum rugula])*.

But how is it now that bacteria always develop in impure materials? In what relation do they stand to putrefaction? To these questions different answers are given.

One says: In the bodies of all living animals and plants the chemical elements are united in a peculiar combination called organic combination. Death loosens the bond by means of which the vital power of the elements was held. This leaves them to the free play of their chemical and physical attractions, and it follows that they rearrange themselves into new and more simple combination, the oxygen at the same time seeking to unite itself with the air, to which certain materials of the dead body have a close affinity. So results separation, disorganization, and new formation, through which the form and organization of the dead body become entirely destroyed. It is this process that we designate putrefaction and decay. It is a purely chemical process, comparable to combustion, effervescence, or the rust of metals. Bacteria find abundant nourishment in the combinations formed by putrefaction, whereas they cannot be nourished on living bodies; no wonder then if their germ, if it find access only singly, increases so extraordinarily by putrefaction.

If this conception be true, bacteria are only chance accompaniers of decay, and the putrefaction of dead bodies must therefore take place under special conditions, even if bacteria are kept away.

These conditions are not easily obtained if we wish to try experiments in order to prove the truth of this supposition. If, for example, we place a piece or some of the juice of an animal or plant, flesh, blood, urine, leaves, fruit, or seeds in a small glass flask, it is most probable that a few of the extraordinarily widespread bacteria will be placed in with it; and this supposition becomes almost a certainty if we add any water to the flask. There is a simple way to banish all the bacteria from the flask, that is, by cooking the same for a long time. Bacteria withstand boiling heat as poorly as other plants and animals; recent experiments have even shown that bacteria are killed by a temperature of 60° C., but the temperature must continue long enough to make sure that the whole mass has been penetrated, and not a single bacterium has escaped destruction. Decay is not removed by cooking alone; experience teaches that cooked flesh, eggs, milk, etc., take a much longer time to decay, but finally they decay, as does raw food.

If one kills the bacteria already in the flask by means of heat, one must take care that no new germs enter from the air. For this purpose, the Italian Abbé Spallanzani, an observer of nature in the last century, celebrated for his shrewd experiments, melted the neck of the little flask together during the cooking; the result was that the animal and plant material enclosed in the flask remained for all time unchanged, without putrefaction.

The French Count N. Appert used this method at the commencement of our century in order to protect meat, vegetables, and other foods; he enclosed them in a metal box furnished with a small opening, then cooked them in a water bath a couple of hours, and during the cooking soldered the opening. Every housewife knows that food will keep for years in metal boxes without spoiling. Certain industries employ this method in preserving food in quantities; we indeed receive beef from Australia, and mutton from America, which is perhaps years old, but when used it is as if fresh.

The assertion has been made that the materials enclosed in the glass flask of Spallanzani, and the metal box of Appert, remained fresh, not because there were no bacteria in them, but rather because there was no oxygen present; for it is certain that by cooking, the air is expelled, and that the entrance of new oxygen through the soldering is impossible. In order to refute this objection, we must allow air free from bacteria to enter the hermetically sealed vessel. To accomplish this. Theodor Schwann in 1837 so modified the experiment of Spallanzani that he did not melt the neck of the flask until after air had been blown in through a red-hot tube. By this process, certainly all the living germs were destroyed.

H. G. F. Schröder and T. Dusch in 1854 used a more convenient method; they stoppered the neck of the little flask with cleansed cotton. After cooking, as the air penetrated into the flask by the cooling of the same, all the germs were retained between the fibers of the cotton, as in a filter.

Finally, in 1863, Pasteur thought of a simpler method. He drew the neck of the flask out, and bent it down horse-shoe shape without melting it; the germs contained in the air, which following gravitation, usually settle down in open vessels, could no longer obtain entrance into the flask.

The result of these methods was always the same; the materials enclosed in the little flasks never fell into putrefaction. Although there was no lack of air, the bacteria found no entrance. From this and many similar experiments, it may be concluded, with the greatest certainty, that where all other conditions for putrefaction obtain, this still does not take place if no bacteria are present. On the contrary, putrefaction begins the moment that bacteria are intentionally or unintentionally added, should this be in the smallest number. Putrefaction proceeds as the bacteria increase; all circumstances which favor the increase of the bacteria accelerate putrefaction; all circumstances which hinder their development delay putrefaction; and all means which destroy bacteria remove putrefaction. On the other hand, the multiplication of bacteria ceases as soon as the substances capable of producing putrefaction are destroyed.

Therefore, bacteria are not the chance companions, but rather the cause, of putrefaction. *Putrefaction is a chemical process, excited by*

bacteria. Death does not, as is generally supposed, cause putrefaction, but rather putrefaction is caused by the life of these invisible organisms.

It appears almost self-evident that every body from which life has departed is subject to corruption; and still it is known that without the life energy of bacteria all bodies after death would retain their form and combination like the Egyptian mummies, or the giants sunk in the Danish moors, or the mammoth and rhinoceros corpses which have remained frozen in the Siberian ice for unnumbered thousands of years and which still retain their skin and hair uninjured. But as soon as the ice melts, these last remains of an extinct animal world sink in a few days to corruption. The cause of this is easily comprehended: The life energy of the bacteria is suspended in the neighborhood of the freezing point, while at a somewhat higher temperature, they immediately multiply and excite putrefaction. In the bog, and in mummies, it is the chemical mixture which hinders the development of the bacteria. When a little piece of flesh or of plant material has remained unchanged for years in a little flask arranged after the method of Spallanzani, Schröder, Dusch, or Pasteur, only a single drop of water containing bacteria need be added in order immediately to start putrefaction.

The whole arrangement of nature is based on this: that the body in which life has been extinguished succumbs to dissolution, in order that its material may become again serviceable to new life. If the amount of material that can be molded into living beings is limited on the earth, the same particles of material must ever be converted from dead into living bodies in an eternal circle; if the wandering of the soul be a myth, the wandering of matter is a scientific fact. If there were no bacteria, the material embodied in animals and plants of one generation would after their decease remain bound, as are the chemical combinations in the rocks; new life could not develop, because there would be a lack of body material. Since bacteria cause the dead body to return to the earth by rapid putrefaction, they alone cause the springing forth of new life; and therefore they make the continuance of living creatures possible. The wonderful fact that putrefaction is a work performed by bacteria is only one of many; an entire series of chemical changes is produced by bacteria and similar microscopic forms. These processes are usually designated as fermentation phenomena, and the organisms which cause the same as fermentation fungi; among the bacteria, the one that has been designated by investigators as *Bacterium termo* is the ferment of putrefaction.

That ferment which has been longest known, and most closely observed, is the alcohol ferment *(Saccharomyces cerevisiae).* Its little oval globules were first observed by Leeuwenhoek in beer, but in 1837 they were recognized by Charles Cagniard de la Tour, and about the same time by Schwann, as the cause of the ferment which changed

sugar into carbonic acid and alcohol, while a small quantity of glycerin and succinic acid were formed. For the closest observations concerning the relation of yeast fungus to alcohol fermentation, we are indebted to Pasteur, from whose fame as the most ingenious and exact of modern French observers we shall not detract, even if he has been so lacking in taste as to carry the bitterness of national passion into the neutral field of science. Pasteur showed that yeast fungus was composed of the same material as all other plants—of carbon, oxygen, hydrogen, and nitrogen, and a number of minerals, among which potash and phosphoric acid are the most important. If the yeast plant is to grow, all these materials must be received as food and applied by its life energy to the building of its cells.

Yeast fungus does not find the whole of its nourishment in clear sugar; it takes some also in the pressed-out juice of grapes, in beer wort, and other fermentables; it multiplies itself only where it finds these. Oxygen and hydrogen are given to it in water; mineral materials which are identified later in the yeast ashes must also be present in solution. It was formerly thought that the yeast plant could take up nitrogen only from albuminous compounds, which are never wanting in juice of grapes and beer wort. Pasteur showed that the need of nitrogen can be satisfied through the taking in of ammonia, which consists of nitrogen and hydrogen. Finally, the yeast plant receives its carbon directly and exclusively from the sugar; probably the albumin present in the cell is formed by a combination of the sugar with the ammonia. Now, since the yeast plant needs the sugar in order to form, nourish, and multiply its particular cells, it causes a dissolution of the sugar and a new arrangement of its finest particles; this gives rise to the change, which has been designated alcohol fermentation. If the fermentation is over, the sugar has also disappeared, and the yeast plant cannot multiply further; it settles itself in the bottom of the fluid as dregs, or is thrown out by the violent escaping of carbonic acid, as foam or yeast, on the top.

Other fermentations are caused by bacteria, or by microscopic organisms which are related to bacteria in that they multiply themselves through division or parting of their cells, and which on that account are united with bacteria in the class *Schizomycetes*. If beer or wine, through time or exposure to the air, becomes sour, acetic acid is formed: It is through bacteria,* which are united in long

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**Mycoderma aceti* [*Acetobacter aceti*], Pasteur. "These little beings reproduce themselves with such rapidity that by placing an imperceptible germ upon the surface of a liquid contained in a vat having a surface of 1 square meter, we may see it covered in from 24 to 48 hours with a uniform velvety veil" (E. Duclaux). This veil is the so-called *mother of vinegar*. The *Mycoderma aceti* must be distinguished from the *Mycoderma vini*, which, while it

chains or bound together in a slimy veil, that the alcohol of spirituous liquors becomes changed to acetic acid. Pasteur has shown that all diseases of wine are caused by microscopic ferment fungi, whose germs get into the fluid during the wine-making and there multiply themselves more or less rapidly. To him belongs the credit of at the same time turning this discovery to the practical account of the wine-makers. When the wine is heated in the flasks to from 50° to 70° C., not only the acetic ferment but also the schizomycetes are killed—all make the wine moldy, slimy, or bitter—and the wine becomes lasting, can be decanted, and increases in fire, bouquet, and value.

When sweet milk becomes sour, it is because the sugar of milk is changed into lactic acid. Here also is a ferment fungus from the class of the bacteria, as Pasteur has proved.* When the milk is cooked, the lactic acid ferment is killed; and if the entrance of new germs is hindered, the milk will remain sweet for an unlimited time. The same lactic acid ferment also plays a part in the preparation of sauerkraut, cucumber pickles, etc. It develops in beet juice and in beer wort, and thus does much injury to the manufacturer.

Other ferment fungi cause other fermentations. One makes urine alkaline,† another changes tannin into gallic acid; still another plays a great role in butyric acid fermentation and in the manufacture of cheese;‡ but especially interesting are the ferment fungi of the

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develops in similar situations, is a species of *Saccharomycete* and is known commonly as *flowers of wine*. "In order that acetification may occur, the oxygen of the air is necessary. Once submerged, the *Mycoderma aceti* develops, but no longer produces acetic acid (Magnin). However: "In effect, it is not then arrested in its work; and without changing form or mode of action, it carries the oxygen of the air to the acetic acid which it has produced, transforming it into carbonic acid and water. If we add some alcohol to the liquid, the phenomena change, the acid is respected, and the alcohol is transformed anew into acetic acid" (Duclaux). *Translator.*

**Vibrio lactis* [*Streptococcus lactis*], Pasteur. Translator.

†*Micrococcus ureae*, Cohn, transforming urea into carbonate of ammonia. *Translator.*

‡*Bacillus subtilis*, Cohn (*Ferment butyrique*, Pasteur). "Butyric fermentation is in fact always preceded by a lactic transformation, and it is by an ulterior modification that the lactic acid produces the butyric acid. The reaction represented by the phenomena is, from a chemical point of view, the following:

$$2C_3H_6O_3 = C_4H_8O_2 + 2CO_2 + 2H_2$$

Lactic Acid Butyric Acid

This fermentation resembles putrefaction in a great many particulars. Indeed some authors include it under the same head" (Magnin). *Translator.*

class of spherical bacteria which originate coloring matter.*

It has long been remarked that suddenly, from time to time, a drop of blood will form on food, and especially on bread, and so increase that it spreads over wide surfaces. This was observed in ancient times, and it was held that it was a sign of threatened disaster, that it showed the anger of God, disclosed secret guilt, and called for bloody atonement; and history records countless sacrifices which, till recent times, were made on account of the superstition, whenever this wonder of blood was seen on food, especially on the consecrated wafer.

With a century of enlightenment, the superstition gradually ceased; but only within the last ten years have we known that the blood wonder had foundation in a fact of science. It was Ehrenberg who first investigated this appearance of blood most carefully. It formed itself in moist air, on cooked but not on raw food; on potatoes, rice, paste, also on flesh, milk, and white of egg--and of itself, without any one voluntarily causing it to be produced. At first it appeared as a very small, rosy red or purple, slimy drop, then grew to the size of a pinhead and looked like fish spawn; then it flattened, ran together, and formed a thick, bloody slime. If one spread out the red drop of gelatin-like substance on a fresh potato, it would multiply rapidly. And it has with ease been increased to so great a quantity that it could be used for coloring; unfortunately this coloring material is not durable, being soon destroyed by the light.

Ehrenberg found in the red slime numberless little oval bodies, to which he gave the name of *Monas prodigiosa;* we designate them better as red spherical bacteria (*Micrococcus prodigiosus* [*Serratia marcescens*]). They nourish themselves on the albumin contained in the food on the surface of which they develop, decompose the food, and generate, by a peculiar pigment fermentation, the red coloring matter, which, as Otto Erdman and J. Schroeter have shown, possesses a striking relation to that brilliant aniline color, which at the present time is of so much value to the coloring industry.

In historical interest, and in the mighty impression it exercised on the myth-forming fancy of the people, the wonder blood is unique; as a physical phenomenon, it takes on a whole series of colors, which

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**Micrococcus chromogenes,* Cohn. "The pigmentary bacteria grow in the state of *Zooglea,* upon the surface of the substances which furnish them with nutriment. They are always alkaline; all are avid for oxygen; their morphological characters are identical, and one can distinguish them only by their different coloring properties.

According to Cohn, they are veritable species: (1) Their pigments offer the greatest diversity as to chemical action, and by spectroscopic analysis, etc. (2) Each species cultivated in the most diverse media produces always the same coloring matter.

They are divided into two categories, according to whether or not the pigment is soluble in water" (Magnin). *Translator.*

appear as a rule in moist places, on potatoes, cheese, cooked eggs, and other foods; in appearance, snow white, sulfur yellow, Spanish green, violet, blue, or brown flecks, little drops or masses of mucus; all these colors, which are also related in part to aniline dyes, originate from spherical bacteria, which, under the microscope, can scarcely be distinguished from the *Micrococcus prodigiosus* of the wonder blood. If milk turns red or yellow of itself, or the pus from a wound takes a Spanish green color, the rod bacteria are designated as the originators of this coloring material.* The litmus so much used by chemists is obtained, together with a few related pigments, from branching or crusty rock growing lichens; the rock is placed in water as long as putrefaction continues, till the at first colorless extract takes on a beautiful purple, red, or blue color wherever it comes in contact with the air. According to recent investigators, it is probable that litmus also is formed through the life energy of bacteria. They have succeeded by means of bacteria in producing, in a clear solution containing cream of tartar and acetate of ammonia, a coloring material exactly similar to litmus, which first colors the fluid a clear blue, becoming from day to day deeper and more beautiful. In other investigations the spherical bacteria appear to a certain extent as manufacturers of Spanish green, yellow, and red coloring materials, which they are able to produce from colorless chemical solutions.

At last, very recently, knowledge of the unexpected secret life energies of bacteria has been revealed, through which they rule with demoniacal power over the weal and woe, and even over the life and death of man.

Probably with the increase of commerce, the visitation of that scourge of God on man and animals, the epidemic, has grown more frequent, in the last ten years. It wanders with uninterruptible progress from city to city, from land to land, stopping at one place but a short time, then, as if exhausted, disappearing in order to carry on its work in a new locality, and usually after an interval of time, turning back again. Only too often the physician's skill and knowledge are exercised in vain to wrest the victim from the devastating power of these diseases, or to limit their course by rules of precaution. As various as are the different forms of disease, yet all epidemics, cholera, pestilence, typhus, diphtheria, variola, scarlatina, hospital gangrene, epizootic, and the like, have certain features in common. These diseases originate nowhere of themselves, neither from internal causes, but are introduced from another place where they have been

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**Bacterium xanthinum*, Schroeter = *Vibrio synxanthus* [*Pseudomonas syncyanea*], Ehrb. *Bacterium syncyanum*, Schroeter = *Vibrio syncyanus* [*Pseudomonas synxantha*], Ehrb. *Bacterium aeruginosum* [*Pseudomonas aeruginosa*], Schroeter. *Translator.*

prevalent, by means of a diseased person or through material which has been in contact with such. They spread only through contagion.

When the infection has taken place, hours or even days may pass before the symptoms appear outwardly. After a certain time of incubation, the disease breaks out through a powerful disturbance of the normal action of all the organs, from the brain to the digestive system; the diseased person appears as if he were under the influence of a poison which had penetrated into his blood; and as he himself is infected by the virus, he spreads it further by the breath, by the perspiration, by the excretions, even in the clothing or the washing. In many diseases the contagious material collects in a concentrated form in peculiar pustules or blisters, whose clear humor, in the slightest quantity, is sufficient to infect a sound person as soon as it has been received into his circulation, and to give him the same appearances of disease as the first victim of the poison. A breeze is sufficient to poison every open wound with the poison that adheres to the knife of the surgeon or anatomist. In anthrax, it is positive that a fly may convey the poison from a diseased to a sound animal.

Scarcely had Leeuwenhoek made known his first observations upon the invisible creatures in rain water, when visionary physicians believed the premature hypothesis that the frightful enigma of epidemics was explained through the wafting of microscopic disease germs. But until the most recent times, every endeavor, even with the help of the microscope, to find with certainty living organisms in the contagious material which originated with the disease, has been in vain. It were as easy to bring to sight the invisible arrow with which, according to the belief of the ancients, Apollo in his anger prostrated men and cattle.

The first discovery of microscopic organisms in contagious diseases we owe to C. Davaine, who in the year 1863 observed in the blood of a splenetic animal, a few hours before its death, numberless fine, thread-like forms, which were almost double the length of blood corpuscles, multiplied themselves by division, and could be distinguished from the ordinary fiber bacteria only through their lack of motion. Davaine on that account designated them *Bacteridia*. Man is subject to a contagious disease which is very nearly related to anthrax,* and in these cases the blood is filled with *Bacteridia*. Within about the past four years the number of epidemics in which bacteria appear has very much increased. This, however, is not the place to discuss separate cases; we shall, therefore, consider some of the more important according to the most recent observations.

Everyone knows how mercilessly diphtheria destroys so many promising lives—an easily transmitted contagion, which usually seats

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**Pustula maligna. Translator.*

itself firmly in the throat and air passages, generating there a membranous formation which threatens immediate death by suffocation. The microscope shows in all the organs of the diseased person innumerable spherical bacteria, heaped together in thick masses, which pass through and besiege the tissues of the muscles, vessels, and mucous membrane; everywhere congestion, inflammation, and a general blood poisoning follow in consequence.

Blood poisoning through open wounds, which in war carries off more victims than the enemies' balls, and which, when it has gained a footing in a hospital, easily causes wounds to become fatal, is always accompanied by the multiplying of the spherical bacteria. These, sometimes singly, sometimes in wreath-like, slimy heaps, settle themselves in the pus or in the tissue of the scar, or are taken up in the blood and deposited in the different organs, where they cause inflammation and the formation of abscesses, and, through a consuming fever, exhaust the most youthful vitality. In the clear lymph of cow or human pox, spherical bacteria are also found in immense numbers, undergoing rapid increase. In the rice water discharges of cholera patients, O. Kolb, as early as 1866, identified numberless bacteria bound together in gelatinous masses. Even the silkworm is subject to an epidemic in which bacteria appear.*

Does it then follow from the presence of bacteria that they truly have to do with epidemics? Is it not quite as probable that these microscopic forms are only chance and unessential accompaniers of disease? Bacteria certainly develop in every fermentation, and where there is any putrefaction, without exerting the least influence on health. As yet, the diffused light of the most recent investigations is not clear enough to enable this dark province to be penetrated; as yet, the newly won ground is not firm enough to warrant the building of an unshakable theory. Still, we already know that the bacteria of contagion are of a different kind from those that cause putrefaction; the former are distinguished from the latter under the microscope mostly through their form; they exist under entirely different circumstances of life; they often battle for existence with the bacteria of putrefaction, and are by them exterminated if they are conquered. C. J. Davaine found that the bacteria of anthrax disappear with the beginning of putrefaction, as early as 48 hours after the death of an animal; and the rod bacteria at the same time multiply beyond measure. But, whereas a drop of blood filled with anthrax bacteria will, when introduced into a sound animal, produce death in from 24 to 36 hours, the inoculation with putrefied blood containing no bacteria is without effect. The bacteria of anthrax do not lose their vitality through desiccation; therefore contagion may take effect through dried blood.

**Micrococcus bombycis*, Cohn. *Translator.*

As is known, only fluid particles can pass through a compact filter, a clay cylinder, or a membrane; little firm bodies, be they ever so small, are stopped. A. Chauveau and E. Klebs made use of these experiments in order to show that in pyemia, septicemia, and variola, the contagion may have its seat, not in the fluid portion of the pus or lymph, but in the microscopic spherical bacteria which develop in it. As they strained this same contagious material through a filter, they ascertained that the clear fluid which had passed through the filter had lost its contagious power, while the firm substances which remained on the filter were efficient.

All these facts make it in the highest degree probable that the already identified bacteria are in many diseases the conveyors and originators of infection, that they are the ferment of contagion. We have the firm conviction that to a more thorough and clearer knowledge of these facts will be joined the discovery of new methods by which to encounter the fearful enemy with better success than hitherto. The skill of the physician should be exerted to answer the following questions: In what way takes place, and in what manner can be hindered, the transportation of microscopic organisms of fermentation? Through what means can the multiplication of the same be arrested? Especially should the physician's attention be turned to water, of which it is ascertained that when in a seemingly pure condition, it is an easy conveyance for the transmission of bacteria and other ferment organisms.

We have seen that bacteria multiply, under favorable circumstances, in all putrefaction and fermentation, and in many diseases, as soon as their germ has once found access; that these smallest of organisms accomplish a vast work because of the immense numbers in which they develop. But from whence is derived the first germ? With this question naturalists have busied themselves up to the present time, and their answers have been various.

One says: By putrefaction, the organic elements which had composed the body of the dead animal, form themselves by free creative power into independent beings, which differ entirely from those from which their material was produced, yet are in every case animated and have the power of propagation; thus the albumin and little fat globules take the form of bacteria, perhaps also of yeast fungus and mold, or even of those little infusorial animals who never fail to be present in corruption. Indeed a special name has been found for this mode of origin, *generatio aequivoca,* or equivocal generation.

Another naturalist disputes the possibility that living beings, however small and simple, ever originated in any other way than from germinal matter which sprang from the same form of life; and he insists that the belief in the equivocal origin of bacteria is the last remnant of an old superstition, which the light of science has not entirely banished. In ancient times it was thought that there originated

from slime, serpents and frogs, which the sun brooded, that caterpillars generated from decayed leaves, vermin from filth, and worms from spoiled meat. Nowadays, every child knows that all these things are fables; every housewife knows by experience that no maggots originate in meat; they do not occur if the blowfly is prevented by a wire screen from entering and depositing its eggs. They have learned, through careful covering, to keep away the minute mold spores, which settle with other dust from the air, and which colonize on their preserved fruits; they know that the trichina and tapeworm originate only from raw or half-cooked pork, in which these animals were already present in the embryonic stage. Even the farmer no longer believes that the grain rust originates from chilling; he knows that it springs from germs which are scattered by the barberry bushes, or other fallen stalks, and that the blight may be arrested in wheat if the seed is strained through sulfate of copper, in order to kill the spores which cling to it.

Concerning bacteria and their related fermentations, the above-mentioned observations lead without doubt to the conclusion that they originate as little through equivocal generation as other living organisms. For when nitrogenous material from the animal and plant world is cooked in flasks, even at as low a temperature as 60° C., all the bacteria are killed; and if the entrance of new germs from outside is in every way hindered, and it were possible to keep the little flask forever, no bacteria would ever originate of themselves. On the contrary the entrance of a single germ is sufficient to cause multiplication, and with it putrefaction. If bacteria originate from putrid material through equivocal generation, putrefaction must appear before bacteria; but experience shows the contrary, that putrefaction is a consequence of the development of bacteria. A theory put forth within the last few years has caused some sensation by seeking to account for the origin of bacteria by saying that the ordinary mold fungus will, under certain conditions, bring forth moving germs of extraordinary minuteness; that these germs are capable of developing into bacteria, into yeast, and finally again into mold. When bacteria are found in the blood or other organs in certain diseases, the authors of this theory are satisfied that the spores of common mold or blight fungus germinate in the human body; that these germs first swarm as bacteria, but under suitable culture may be nourished into different kinds of mold. However, unprejudiced research has not given the slightest proof that bacteria stand in any connection with the history of the development of yeast, blight, or mold fungus. They always originate, as far as we know at present, from germs of the same kind.

Through these facts, we surely have a right to hope that in the development of bacteria the key will be found to the origin of life in the world in general. If we could prove that through equivocal generation, one single organism, or living cell, shaped itself from

unorganized and lifeless material, then could we conceive that the first created beings were in the beginning formed in a similar manner.

Now it is very certain that life had a beginning on the earth, but how did the first living beings originate? For this, all analogy is wanting. According to our present knowledge, life may be compared to the holy fire of Vesta, which is eternally maintained, through the kindling of the new brand from the old.

The famous physicist Wyville Thompson, in an ingenious speech with which he opened the Natural Science Convention in Edinburgh, drew the following conclusions: that life on this earth, not having originated of itself, must therefore have been conveyed to our world from another. We know that the numberless meteoric stones which have fallen to the earth were once independent bodies or parts of such. In certain meteorites, carbon, and certain combinations containing carbon, have been found, which points to organic formation. It is possible to think that, at some time, a germ, with life and capacity for development, could have survived the glowing heat which generally accompanies the entrance of a newcomer from space into our atmosphere, and that from such a germ all living beings might have descended. Thus, at some time the commencement of life may have descended from Heaven upon this lifeless earth, as, according to the myth, the living spark was brought down by Prometheus from Olympus.

The developmental cycle of the bacteria allows us to think of another origin of life on the earth. We have calculated the weight of one bacterium at 0.000,000,001,57 mg.; we know that these infinitely light little bodies are carried away through the evaporation of water, and float around in the air as little particles of sun dust, and with the dust again settle down; but they may be carried by the winds to unmeasurable distances, and also to extraordinary heights. It is more probable that these particles of fine dust are sometimes carried so far up by ascending currents of air that they are deprived of the attraction of our planet, and reach space. The existence of a world dust is presumable from certain cosmical appearance of light. Space is exceedingly cold, yet experiments prove that even a chilling for many hours below -18° C. does not kill bacteria; they become stiff through cold, from which condition they revive when thawed, and under favorable circumstances again begin to multiply.* It is perhaps not impossible that an ascending particle of bacteria dust, which has floated for a long time in space, may reach the atmosphere of another world; and if it find there circumstances favorable to life, it multiplies.

On the other hand, it is possible to think that a germ of *Bacterium*, or any other exceedingly small and simple form, from some other life-nourishing world, may have been moving about in space, and that such

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*See previous note in regard to spores. *Translator.*

a germ, finally reaching our atmosphere, settled to the earth. As long as the primitive sea retained a temperature above 60° C., the development of such a germ would have been impossible; but as soon as the cooling had fallen below this temperature, the stray germ of life must have found in the sea, richly saturated with salt, conditions for unlimited multiplication. We have calculated that the whole ocean might be filled with such organisms in a few days.

From these first living germs, in which the characteristics of the animal and vegetable kingdoms are not yet separated, the laws of development, the battle for existence, natural increase, geographical distribution, and many other known and unknown forces might have produced the different forms of the animal and plant world, which inhabited the earth in the past as they do in the present. We know well that in the maintenance of such views, we stray far from the boundaries of natural philosophy; but we find the naturalist, always remaining conscious of the limitation of his knowledge, admitting his ignorance with resignation, foiled in his experiments and observations; not always resisting the longing of Faust, "Zu schauen alle Wirkungskraft und Samen," but gladly giving himself up to the allurement of filling with some fantasy that blank at which modern investigation has failed.

Further Reading

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V.

JOHN TYNDALL

(1820 to 1893)

John Tyndall's early years found him employed in a variety of jobs before he turned his attention to physics; however, in 1851, after only a few years of concentrated study, he obtained his doctorate in physics from the University of Marburg with a dissertation on screw surfaces. He became known through his work on magnetism, and was elected to the Royal Society of London in 1852, when he was only thirty-two. In 1854 he was appointed professor of natural philosophy at the Royal Institution of Great Britain, and thereby became a colleague of Michael Faraday, whom he succeeded as superintendent in 1867. In 1868 he wrote a memorial volume, *Faraday As a Discoverer*. Tyndall was born on August 2d, 1820, at Leighlin Bridge, County Carlow, Ireland; and he died at Hindhead, England, on December 4th, 1893, after being accidentally poisoned.

Tyndall's work was mainly in the realm of physics, and he published books on heat (1863), sound (1867), and light (1873). In 1872 and 1873, Tyndall presented a series of lectures in the United States in which he discussed his work. Characteristic of him, he donated the profits ($14,000) from the venture to a fund for the advancement of American science.

Tyndall devoted considerable time (1859-69) to studying radiant heat and gases, and during the course of this work he discovered that when a beam of light was projected through an "optically inactive", that is, dust-free space, it became invisible. His interest in air-borne bacteria was first made known in 1870, and he entered into the controversy over the spontaneous generation of microorganisms following an attack on his views by H. C. Bastian, the English heterogenist. Thereafter, Tyndall inaugurated a series of brilliant experiments, many of which were conceived from a physicist's point of view, and which were summarized in his *Essays on the Floating Matter of the Air in Relation to Putrefaction and Infection* (1881).

In addition to dealing devastating blows to proponents of the doctrine of spontaneous generation, Tyndall contributed much to our

knowledge of the heat resistance of bacterial spores; and he should be credited with drawing the attention of medical men to the significance of his results in their own work. In fact, this latter point was appreciated by the University of Tübingen, in that it conferred on Tyndall an honorary doctorate of medicine.

Tyndall's bacteriological investigations have not yet been accorded the recognition they deserve, although fractional sterilization came to be designated "tyndallization." A gifted lecturer, Tyndall did much, as did his fellow Britisher, Joseph Lister, to make current Pasteur's doctrines in England.

Curiously, two of the men who had much to do with the banishment of spontaneous generation doctrines, and the establishment of microbiology as a science, came into their work from the physical sciences. Pasteur arrived by way of chemistry, and Tyndall by way of physics. Whereas the former noted the effects of contaminated dust particles of the air by the chemical alteration of infusions and solutions, the latter noted from physical studies that "optically empty" air was sterile air. Pasteur's results and explanations were considerably strengthened by Tyndall's experiments. It is obvious that the latter's early work on the physical properties of gases and vapors prepared him for this task.

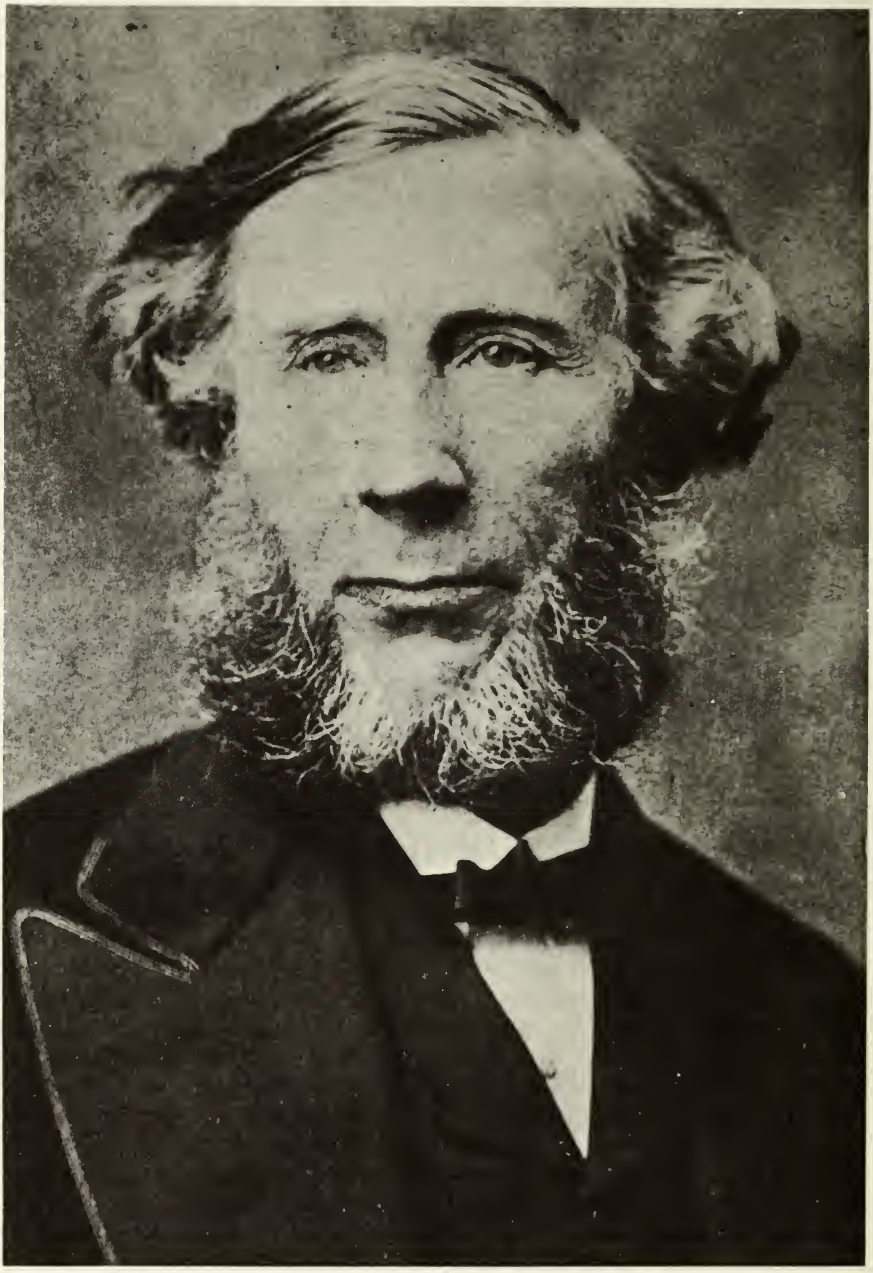
The following excerpt is from a long paper presented before the Royal Society of London on January 13th, 1876. It was published later the same year in the Society's *Philosophical Transactions,* CLXVI (1876), 27-74.

THE OPTICAL DEPORTMENT OF THE ATMOSPHERE IN RELATION TO THE PHENOMENA OF PUTREFACTION AND INFECTION

An inquiry into the decomposition of vapors by light, begun in 1868 and continued in 1869, in which it was necessary to employ optically pure air, led me to experiment on the floating matter of the atmosphere. A brief section of a paper published in the *Philosophical Transactions* for 1870[1] is devoted to this subject.

I at that time found that London air, which is always thick with motes, and also with matter too fine to be described as motes, after it had been filtered by passing it through densely packed cottonwool,

[1] *Proceedings of the Royal Society of London,* CLX, 337.



National Library of Medicine

John Tyndall

or calcined by passing it through a red-hot platinum tube containing a bundle of red-hot platinum wires, or by carefully leading it over the top of a spirit-lamp flame, showed, when examined by a concentrated luminous beam, no trace of mechanically suspended matter. The particular portion of space occupied by such a beam was not to be distinguished from adjacent space.

The purely gaseous portion of our atmosphere was thus shown to be incompetent to scatter light.

I subsequently found that to render the air thus optically pure, it was only necessary to leave it to itself for a sufficient time in a closed chamber, or in a suitably closed vessel. The floating matter gradually attached itself to the top and sides, or sank to the bottom, leaving behind it air possessing no scattering power. Sent through such air, the most concentrated beam failed to render its track visible.

I mention "top" and "sides," as well as "bottom," because gravity is not the only agent, probably not the principal agent, concerned in the removal of the floating matter. It is practically impossible to surround a closed vessel by an absolutely uniform temperature; and where differences of temperature, however small, exist, air currents will be established. By such gentle currents the floating particles are gradually brought into contact with all the surrounding surfaces. To these they adhere, and, no new supply being admitted, the suspended matter finally disappears from the air altogether.

The parallelism of these results with those obtained in the excellent researches of T. Schwann, H. G. F. Schröder and T. Dusch, Schröder himself, and L. Pasteur in regard to the question of "spontaneous generation," caused me to conclude that the power of scattering light and the power of producing life by the air would be found to go hand in hand.

This conclusion was strengthened by an experiment easily made and of high significance in relation to this question. It had been pointed out by Professor Joseph Lister, of Edinburgh,[2] that air which has passed through the lungs is known to have lost its power of causing putrefaction. Such air may mix freely with the blood without risk of mischief; and that truly great scientific Surgeon had the penetration to ascribe this immunity from danger to the filtering power of the lungs. Prior to my becoming acquainted with this hypothesis in 1869, I had demonstrated its accuracy in the following manner.

Condensing in a dark room, and in dusty air, a powerful beam of light, and breathing through a glass tube (the tube actually employed was a lamp glass, rendered warm in a flame to prevent precipitation) across the focus, a diminution of the scattered light was first observed. But towards the end of the expiration the white track of the

[2] Introductory lecture before the University.

beam was broken by a perfectly black gap, the blackness being due to the total absence from the expired air of any matter competent to scatter light. The experimental arrangement is represented in Figure 1* where g represents the heated lamp glass, and b the gap cut out of the beam issuing from the lamp L. The deeper portions of the lungs were thus proved to be filled with optically pure air, which, as such, had no power to generate the organisms essential to the process of putrefaction.[3]

It seemed that this simple method of examination could not fail to be of use to workers in this field. They had hitherto proceeded less by sight than by insight, being in general unable to see the physical character of the medium in which their experiments were conducted. But the method has not been much turned to account; and this year I thought it worth while to devote some time myself to the more complete demonstration of its utility.

I also wished to free my mind, and if possible the minds of others, from the uncertainty and confusion which now beset the doctrine of "spontaneous generation." Pasteur has pronounced it "a chimera," and expressed the undoubting conviction that this being so, it is possible to remove parasitic diseases from the earth. To the medical profession, therefore, and through them to humanity at large, this question, if the illustrious French philosopher be correct, is one of the last importance. But Pasteur's labors, which have so long been considered models by most of us, have been subjected to rough handling of late. His reasoning has been criticized, and experiments counter to his have been adduced in such number and variety, and with such an appearance of circumstantial accuracy, as to render the evidence against him overwhelming to many minds. This

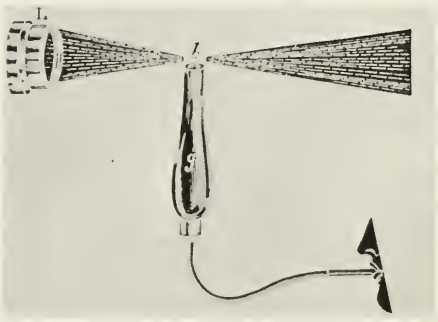


Figure 1

[3] "No putrefaction", says Ferdinand Cohn, "can occur in a nitrogenous substance if it be kept free from the entrance of new *Bacteria* after those which it may contain have been destroyed. Putrefaction begins as soon as *Bacteria*, even in the smallest numbers, are accidentally or purposely introduced. It progresses in direct proportion to the multiplication of the *Bacteria;* it is retarded when the *Bacteria* (for example, by a low temperature) develop a small amount of vitality, and is brought to an end by all influences which either stop the development of the *Bacteria*, or kill them. All bactericidal media are therefore antiseptic and disinfecting." *Beiträge zur Biologie der Pflanzen*, II (1872), 203.

*Tyndall's figures are reproduced by courtesy of the Royal Society of London and D. C. Martin. *Editor.*

I have reason to know, has been the effect wrought, not only upon persons untrained in science, but also upon biologists of eminence both in this country and America.

The state of medical opinion in England is correctly described in a recent number of the *British Medical Journal,* where, in answer to the question, "In what way is contagium generated and communicated?" we have the reply that, notwithstanding "an almost incalculable amount of patient labor, the actual results obtained, especially as regards the manner of generation of contagium, have been most disappointing. Observers are even yet at variance whether these minute particles, whose discovery we have just noticed, and other disease germs, are always produced from like bodies previously existing, or whether they do not, under certain favorable conditions, spring into existence *de novo.*"

With a view to the possible diminution of the uncertainty thus described, I beg without further preface to submit to the Royal Society, and especially to those who study the etiology of disease, the following description of the mode of procedure followed in this inquiry, and of the results to which it has led.

Method of Experiment

A chamber, or case, was constructed, with a glass front, its top, bottom, back, and sides being of wood. At the back is a little door which opens and closes on hinges, while into the sides are inserted two panes of glass, facing each other. The top is perforated in the middle by a hole 2 in. in diameter, closed air-tight by a sheet of India rubber. This sheet is pierced in the middle by a pin, and through the pin hole is passed the shank of a long pipette ending above in a small funnel. A circular tin collar, 2 in. in diameter and $1\frac{1}{2}$ in. deep, surrounds the pipette, the space between both being packed with cotton wool moistened by glycerin. Thus the pipette, in moving up and down, not only is firmly clasped by the India rubber, but it also passes through a stuffing box of sticky cotton wool. The width of the aperture closed by the India rubber secures the free lateral play of the lower end of the pipette. Into two other smaller apertures in the top of the cupboard are inserted, air-tight, the open ends of two narrow tubes, intended to connect the interior space with the atmosphere. The tubes are bent several times up and down, so as to intercept and retain the particles carried by such feeble currents as changes of temperature might cause to set in between the outer and the inner air.

The bottom of the box is pierced with two rows of holes, six in a row, in which are fixed, air-tight, twelve test tubes, intended to contain the liquid to be exposed to the action of the moteless air.

The arrangement is represented in Figure 2, where *ww* are the side windows through which the searching beam passes from the lamp

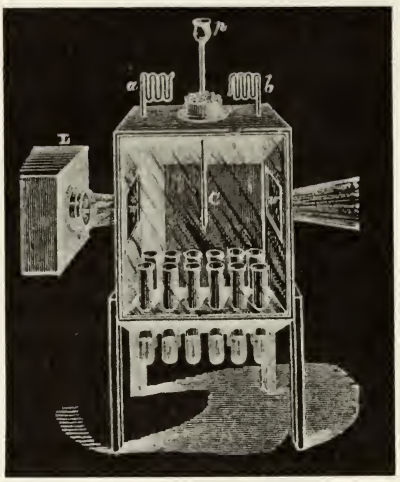


Figure 2

L across the case *C; p* is the pipette, and *a* and *b* are the bent tubes connecting the inner and outer air. The test tubes passing through the bottom of case are seen below.

On September 10th this case was closed. The passage of a concentrated beam across it through its two side windows then showed the air within it to be laden with floating matter. On the 13th it was again examined. Before the beam entered, and after it quitted the case, its track was vivid in the air, but within the case it vanished. Three days of quiet sufficed to cause all the floating matter to be deposited on the interior surfaces, where it was retained by a coating of glycerin, with which these surfaces had been purposely varnished.

Deportment of Urine

The pipette being dipped into the tubes, fresh urine was poured into eight of them in succession on last September 13th. Each tube was about half-filled with the liquid. The tubes were then immersed in a bath of brine, raised to ebullition, and permitted to boil for five minutes. Aqueous vapor rose from the liquid into the chamber, where it was for the most part condensed, the uncondensed portion escaping, at a low temperature, through the bent tubes at the top. Before the brine was removed, little stoppers of cotton wool were inserted in the bent tubes, lest the entrance of the air into the cooling chamber should at first be forcible enough to carry motes along with it. As soon, however, as the ambient temperature was assumed by the air within the case, the cotton wool stoppers were removed.

The front and back of this chamber were squares of 14 in. the side, the depth of the chamber being 8.5 in. It contained, therefore, 1,666 cu. in. of air, which had unimpeded access to the liquid in the tubes. No stoppers were employed. The air was unaffected by calcination, or even by filtering. Neither cotton wool nor hermetic sealing was resorted to. Self-subsidence was the only means employed to rid the "untortured" air of its floating matter.

A second series of eight tubes were filled at the same time with the same liquid, and subjected to the same boiling process. The only difference between the two series was that these latter tubes were placed in a stand beside the former and exposed to the common air of the laboratory.

For the sake of distinction I will call the tubes opening into the case the *protected* tubes, and those opening into the common air the *exposed* tubes.

On September 17th all the protected tubes were bright and clear, while all the exposed tubes were distinctly turbid. Specks of mold, moreover, were in every case seen on the surface of the exposed liquid. These waxed daily larger, and finally formed a thick layer on the top of every column. The liquid changed from a pale sherry to a reddish-brown color, some of the tubes being more deeply tinged than others.

On September 27th I provided myself with a microscope having a magnifying power of 1,200 diameters. Under its scrutiny the turbidity of the liquid immediately resolved itself into swarms of *Bacteria* in active motion. F. Cohn correctly explains the turbidity. The index of refraction of the *Bacterium* being slightly different from that of the surrounding medium, a scattering of light is the consequence. This scattering, however, and the opalescence it produces, are practically independent of the motions of the *Bacteria*.

Since the date here referred to, the exposed liquid has been frequently examined, both with the eye and with the microscope. To the former it is thickly turbid, to the latter it is swarming with life. Its smell is putrid. *All this time the protected tubes exhibit a liquid perfectly unchanged in appearance.* For four months it has remained as transparent and of as rich a color as the brightest Amontillado sherry.

On October 1st another experiment similar in principle to that just described was begun. Fresh urine was employed, and a much smaller case. The capacity of the latter was 451 cu. in.; and three test tubes, instead of twelve, were passed airtight through its bottom. Like those in the larger chamber, they were filled by a pipette, and boiled for five minutes in a bath of brine. Beside them were placed three other tubes containing the same liquid treated in exactly the same way, but exposed to the common air. On the 5th all the exposed tubes were turbid, and found by microscopic examination to be swarming with *Bacteria*. The color of the exposed liquid changed from a pale sherry color to a brown-orange. On the 25th the tubes were again examined, and found full of *Bacteria*. Two months subsequent to this latter date, the infusion, diminished by evaporation, was found well charged with bacterial life.

While this process of putrefaction was going on outside, the tubes opening into the moteless air of the case remained perfectly clear.

The chamber represented in Figure 2, and above described, was

the first operated on, and the liquid is shown by the draftsman as filling only a small portion of the test tubes. This smallness of volume is in part due to evaporation. Test tubes 1.2 in. wide and 9 in. long were, in all subsequent experiments, nearly filled with the infusions. Strong in the first instance, these were sometimes kept until slow evaporation through the open tubes at the top of the case had reduced them to one-third or one-fourth of their original volume. Each experiment, therefore, was, in reality, a series of experiments, extending over months, on infusions of different strengths, the concluding ones of the series attaining a very high degree of concentration. In Figure 2 the portion of the tubes within the case ought to be less than one-half of what it is there shown to be.

The next eleven sections of this paper are not presented here. In these, Tyndall further developed his technique by making observations on infusions of mutton, beef, hay, liver, tea, coffee, hops, and a number of other common household materials. Editor.

Partial Discussion of the Results

Thus by experiments, reiterated in many cases, with urine, mutton, beef, pork, hay, turnip, tea, coffee, hops, haddock, sole, salmon, codfish, turbot, mullet, herring, eel, oyster, whiting, liver, kidney, hare, rabbit, fowl, pheasant, grouse, has the induction been established that the power of developing bacterial life by atmospheric air, and its power of scattering light, go hand in hand. We shall immediately examine more closely what this means.

In his published works, H. C. Bastian has frequently dwelt upon the necessity of employing strong infusions when investigating the phenomena of spontaneous generation. I would therefore recall to mind what has been stated on a previous page, that in most of the experiments here described the infusion at starting was strong, and that it was permitted to evaporate with extreme slowness until its concentration became three- or four-fold what it has been at starting. Every experiment was thus converted into an indefinite number of experiments on infusions of different strengths. Never, in my opinion, was the requirement as to concentration more completely fulfilled, and never was the reply of Nature to experiment more definite and satisfactory. The temperatures, moreover, to which the infusions have been subjected embrace those hitherto found effectual, extending indeed beyond them in both directions. They reached from a lower limit of 50° to a higher limit of more than 100° F. Still higher temperatures were applied in other experiments to be described subsequently. With regard to the number of the infusions, more than fifty moteless chambers, each with its system of tubes, have been tested. There is no shade of uncertainty in any of the results. In every instance we have, within the chamber, perfect limpidity and sweetness—

without the chamber, putridity and its characteristic smells. In no instance is the least countenance lent to the notion that an infusion deprived by heat of its inherent life, and placed in contact with air cleansed of its visibly suspended matter, has any power whatever to generate life anew.

If it should be asked how I have assured myself that the protected liquids do not contain *Bacteria,* I would, in the first place, reply that with the most careful microscopic search I have been unable to find them. But much more than this may be affirmed. The electric or the solar beam is a far more powerful and searching test in this matter than the microscope. In the foregoing pages I have more than once described the clearness of my protected infusions, after months of exposure, as equal to that of distilled water. So far is this from being an exaggeration, that it falls short of the truth; for I have never seen distilled water so free from suspended particles as the protected infusions prove themselves to be. When for months a transparent liquid thus defies the scrutiny of the searching beam, maintaining itself free from every speck which could scatter light as a *Bacterium* scatters it —when, moreover, an adjacent infusion, prepared in precisely the same way, but exposed to the ordinary air, becomes first hazy, then turbid, and ends by wholly shattering the concentrated beam into irregularly scattered light, I think we are entitled to conclude that *Bacteria* are as certainly absent from the one as they are present in the other.

For the right interpretation of scientific evidence, something more than mere sharpness of observation is requisite, very keen sight being perfectly compatible with very weak insight. I was therefore careful to have my infusions inspected by biologists, not only trained in the niceties of the microscope, but versed in all the processes of scientific reasoning. Their conclusion is that it would simply weaken the demonstrative force of the experiments to appeal to the microscope at all.

Suspended Particles in Air and Water— Their Relation to Bacteria

Examined by the concentrated solar rays, or by the condensed electric beam, the floating matter of the air is seen to consist; first, of particles so coarse that their individual motions can be followed by the eye; second, of a finer matter which is not to be distinguished as motes, but which emits a uniform and changeless light. In this finer matter the coarser motes move as in a medium. . . .

When the track of a parallel beam in dusty air is looked at horizontally through a Nicol's prism, in a direction perpendicular to the beam, the longer diagonal of the prism being vertical, a portion of the light from the finer matter, being polarized, is extinguished. The

coarser motes, on the other hand, which do not polarize the light, flash out with greater force, because of the increased darkness of the space around them.

The individual particles of the finest floating matter of the air lie probably far beyond the reach of the microscope. At all events it is experimentally demonstrable that there are particles which act similarly upon light, and which are entirely ultra-microscopic. A few days ago, for example, an inverted bell jar was filled with distilled water, into which, while it was briskly beaten by a glass rod, was dropped a solution of mastic in alcohol. The proportion was less than that employed by Brücke, being about 10 gr. of the gum to 1,000 gr. of the alcohol. The jar was placed under a skylight, at the height of the eye above the floor. It was of a beautiful cerulean hue, this color arising wholly from the light scattered by the mastic particles. Looked at horizontally through a Nicol's prism, with its shorter diagonal vertical, the blue light passed freely to the eye. Turning the long diagonal vertical, the scattered light was wholly quenched, and the jar appeared as if filled with ordinary pure water.

I tried the effect of a powerful filter upon those particles, and found that they passed sensibly unimpeded through forty layers of the best filtering paper.[4]

The liquid containing them was examined by a microscope magnifying 1,200 diameters. The suspended mastic particles entirely eluded this power, the medium in which they swam being as uniform as distilled water in which no mastic whatever had been precipitated.

The optical deportment of the floating matter of the air proves it to be composed, in part, of particles of this excessively minute character. The concentrated beam reveals them collectively, long after the microscope has ceased to distinguish them individually. They are, moreover, organic particles, which may be removed from the air by combustion. In presence of such facts, any argument against atmospheric germs, based upon their being beyond the reach of the microscope, loses all validity.

We are here brought face to face with a question of extreme importance, which it will be useful to clear up. "Potential germs" and "hypothetical germs" have been spoken of with scorn, because the evidence of the microscope as to their existence was not forthcoming Sagacious writers had drawn from their experiments the perfectly legitimate inference that in many cases the germs exist, though the microscope fails to reveal them. Such inferences, however, have been treated as the pure work of the imagination, resting, it was alleged, on no real basis of fact. But in the concentrated beam we possess what is virtually a new instrument, exceeding the microscope indefinitely in power. Directing it upon media which refuse to give the coarser

[4] There are filters, however, which stop them; but of this immediately.

instrument any information as to what they hold in suspension, these media declare themselves to be crowded with particles--not hypothetical, not potential, but actual and myriadfold in number—showing the microscopist that there is a world beyond even his range.

In sections 6 and 8,* experiments on the infection of clear infusions by others containing visible *Bacteria* are referred to. But for the infection to be sure, it is not necessary that the *Bacteria* should be visible. Over and over again I have repeated the experiments of Burdon Sanderson on the infective power of ordinary distilled water, in which the microscope fails to reveal a *Bacterium*. The water, for example, furnished to the Royal Institution laboratory is sensibly as infectious as an infusion swarming with *Bacteria*.

Perhaps the severest experiment of this kind ever made was one executed by Dr. Sanderson with water prepared by myself. In 1871 I sought anxiously and assiduously for water free from suspended particles. The liquid was obtained in various degrees of purity, but never entirely pure. Knowing the wonderful power of extrusion, as regards foreign matter, brought into play by water in crystallizing, the thought occurred to me of examining the liquid derived from the fusion of the most transparent ice.

Mr. J. Cottrell, at my request, arranged the following apparatus for me: Through the plate of an air pump (Figure 3) passed air-tight the shank of a large funnel. A small glass bulb, *B*, furnished with a glass stopcock, was attached to the shank of the funnel below. Prior to being put together, all parts of the apparatus had been scrupulously cleansed. In the funnel was placed a block of ice, *I*, selected for its transparency, having a volume of 1,000 cu. in. or thereabouts, and over the ice was placed an air-tight receiver. Several times in succession the air was removed from this receiver, its place on each occasion being taken by other air carefully filtered through cotton wool. The transparent ice was thus surrounded by moteless air.

The ice was now permitted to melt; its water trickled into the small glass bulb below, which was filled and emptied a great number of times. From the very heart of the block of ice the water was finally taken and subjected to the scrutiny of the concentration beam. It proved to be the purest liquid I had ever seen—probably the purest human eye had ever seen; but still it contained myriads of ultramicroscopic particles. The track of the beam through it was of the most delicate blue, the blue light being perfectly polarized. It could be wholly quenched by a Nicol's prism, the beam then passing through a vacuum. A comparison of the light with that scattered by such mastic particles as those above referred to, proved the suspended particles of the ice water to be far smaller than those of the mastic. No microscope, therefore, could come near them. Such water, however,

*Deleted from this presentation. *Editor.*

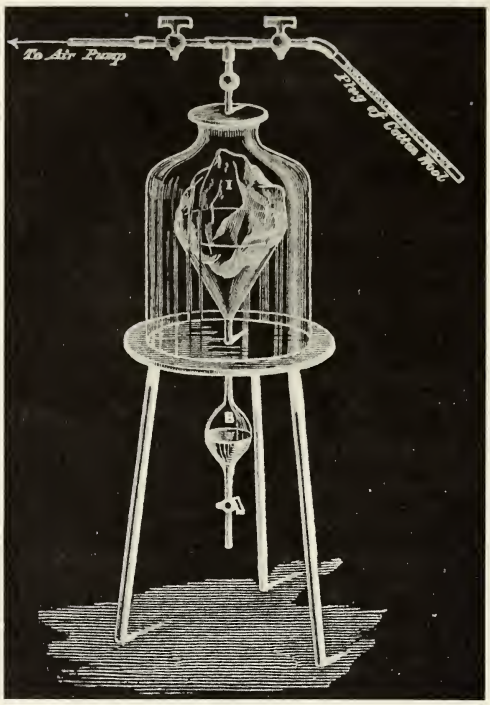


Figure 3

was proved by Dr. Sanderson to be as infectious as the water from any ordinary tap.

Infinitesimal as these particles are, however, they may be separated by mechanical means from the liquid in which they are held in suspension. Filters of porous earthenware, such as the porous cells of Bunsen's battery, have been turned to important account in the researches of F. W. Zahn, E. Klebs, and Burdon Sanderson. In various instances it has been proved that, as regards the infection of living animals, the porous earthenware intercepts the contagium. For the living animal, organic infusions or Pasteur's solution may be substituted. Not only are ice water, distilled water, and tap water thus deprived of their powers of infection, but by plunging the porous cell into an infusion swarming with bacterial life, exhausting the cell, and permitting the liquid to be slowly driven through it by atmospheric pressure, the filtrate is deprived not only of its *Bacteria* but also of those ultra-microscopic particles which appear to be as potent for infection as the *Bacteria* themselves. The precipitated mastic particles before described, which pass unimpeded through an indefinite number of paper filters, are wholly intercepted by the porous cell.

These germinal particles abound in every pool, stream, and river. All parts of the moist earth are crowded with them. Every wetted surface which has been dried by the sun or air contains upon it the particles which the unevaporated liquid held in suspension. From such surfaces they are detached and wafted away, their universal prevalence in the atmosphere being thus accounted for. Doubtless they sometimes attach themselves to the coarser particles, organic and inorganic, which are left behind along with them; but they need no such rafts to carry them through the air, being themselves endowed

with a power of flotation commensurate with their extreme smallness and the specific lightness of the matter of which they are composed.

I by no means affirm that the developed *Bacterium,* which requires for its maintenance nutriment beyond that which ordinary water can always supply, is never wafted through the air. Cases doubtless will arise favorable for the growth and dispersion of the full-grown organism. Whether, after desiccation, it retains the power of reproduction is another question. But it ought, I think, to be steadily borne in mind that the *Bacteria* and the atmospheric matter from which they are developed are, in general, different things. I have carefully sought for atmospheric *Bacteria,* but have never found them. They have never, to my knowledge, been found by others; and that they arise from matter which has not yet assumed the bacterial form is, as just shown, capable of demonstration. An organic infusion, boiled and shielded from atmospheric particles, will remain clear for an indefinite period, while a fragment of grass which has been exposed to the air, but on which no trace of a *Bacterium* is to be found, will in two or three days develop in it a multitudinous crop of life.

We have now to look a little more closely at these particles, foreign to the atmosphere but floating in it, and proved beyond doubt to be the origin of all the bacterial life which our experiments have thus far revealed. We must also look at them as they exist in water, in countless multitudes, being as foreign to this medium as the floating atmospheric dust is to the air in which it swims. The existence of the particles is quite as certain as if they could be felt between the fingers, or seen by the naked eye, Supposing them to augment in magnitude until they come, not only within range of the microscope, but within range of the unaided senses. Let it be assumed that our knowledge of them under these circumstances remains as defective as it is now—that we do not know whether they are germs, particles of dead organic dust, or particles of mineral matter. Suppose a vessel (say a flower pot) to be at hand filled with nutritious earth, with which we mix our unknown particles, and that in 48 hours subsequently buds and blades of well-defined cresses and grasses appear above the soil. Suppose the experiment when repeated a hundred times to yield the same unvarying result. What would be our conclusion? Should we regard those living plants as the product of dead dust, of mineral particles? or should we regard them as the offspring of living seeds? The reply is unavoidable. We should undoubtedly consider the experiment with the flower pot as clearing up our pre-existing ignorance; we should regard the fact of their producing cresses and grasses as proof positive that the particles sown in the earth of the pot were the seeds of the plants which have grown from them. It would be simply monstrous to conclude that they had been "spontaneously generated."

This reasoning applies word for word to the development of *Bacteria* from that floating matter which the electric beam reveals in the

air, and in the absence of which no bacterial life has been generated. I cannot see a flaw in the reasoning; and it is so simple as to render it unlikely that the notion of bacterial life developed from dead dust can every gain currency among the members of the medical profession.

It has been said of those whom the evidence adduced in favor of spontaneous generation fails to convince, that they seem willing to believe in almost any infringement of natural uniformity rather than admit the doctrine. This surely is an inversion of the true order of the facts. Natural uniformity is the record of experience; and, apart from the phenomena to be accounted for, there is not a vestige of experience, possessed by either the man of science or the human race, which warrants the notion that dead dust, and not living seed, is the source of the crops which spring from our infusions after their impregnation by the floating particles of the atmosphere.

Further Readings

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VI.

ROBERT KOCH

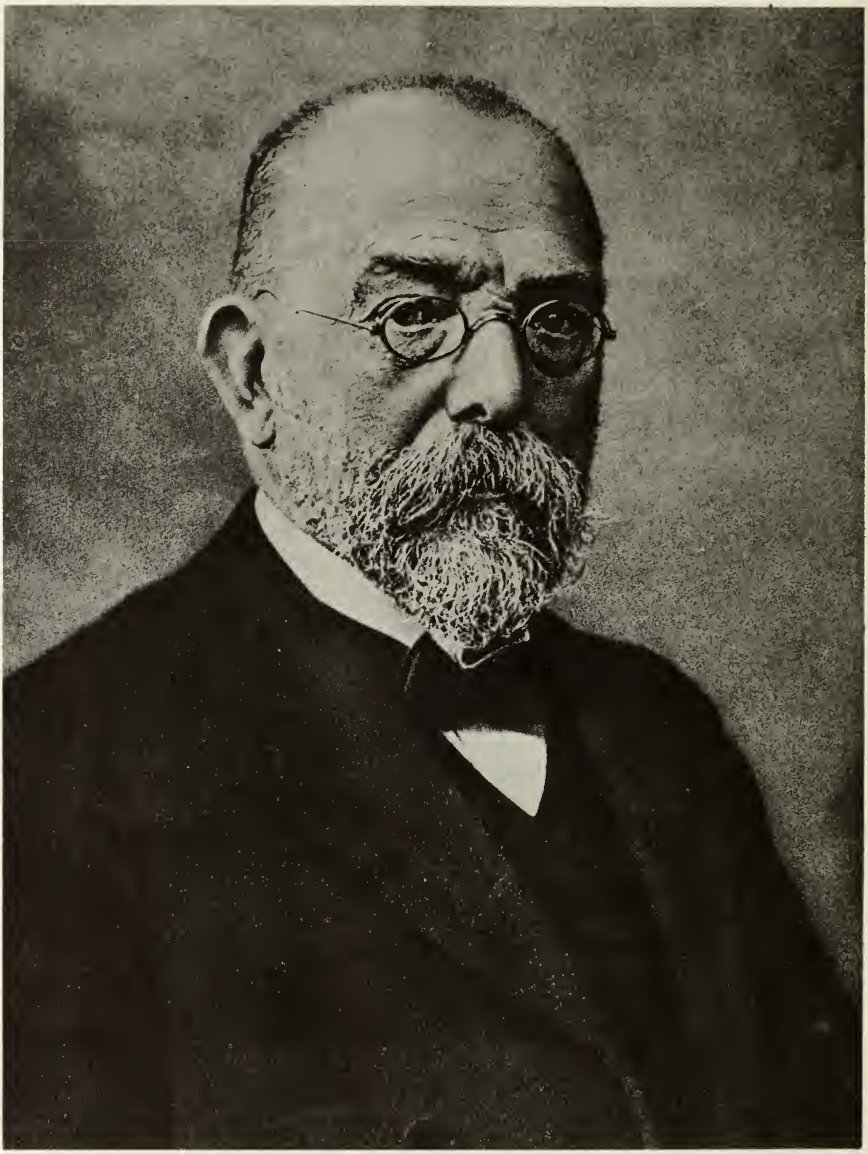
(1843 to 1910)

Robert Koch was born in Clausthal, Germany, on December 11th, 1843, and he died in Baden-Baden on May 28th, 1910. He obtained his medical degree from Göttingen after studies under F. G. J. Henle and F. Wöhler. He was a medical officer in Wollstein from 1872 to 1876 and during these few years completed his work on growing the anthrax bacillus in pure culture (1876). Thereafter Koch became famous for his contributions to all phases of the new science of bacteriology. In 1882 he isolated the tuberculosis organism, and in 1883 the vibrio of cholera. He founded the great bacteriological school at Berlin, which attracted students from all over the world, and from 1891 to 1904 he was director of the Institute of Infectious Diseases in Berlin.

Koch traveled extensively during his lifetime in his efforts to find the cause and control of diseases. In 1896 he went to South Africa to study rinderpest, in 1897 to Bombay to study bubonic plague, and in 1906-07 to East Africa to study sleeping sickness.

Koch received the Nobel prize in medicine in 1905 for his work on tuberculosis. The citation reads as follows: "Seldom has an investigator been able with such perspicacity to envisage in advance an uncharted region of research, and seldom has one been so brilliant and successful in the development thereof, as has Robert Koch. Seldom, however, have so many discoveries, of incisive importance for the human race, emanated from the activity of a single individual, as has been the case with him."

Koch was a master of bacteriological technique. The paper presented here shows him at his best in solving some basic problems of a new science. In fact, it will be recognized by the reader that many of the techniques used today in the bacteriology laboratory are merely refinements of Koch's original methods. Koch's paper is an excerpt of a paper entitled "Verfahren zur Untersuchung, zum Conservieren und Photographieren der Bacterien." published in Ferdinand Cohn's *Beiträge zur Biologie der Pflanzen,* II (1877), 399-434.



National Library of Medicine

Robert Koch

METHODS FOR STUDYING, PRESERVING, AND PHOTOGRAPHING BACTERIA

There has been considerable improvement in the last year in methods for studying bacteria. C. Weigert has contributed a perfected hematoxylin staining method for demonstrating the distribution of bacteria in animal tissues. C. J. Salomonsen has presented a unique culture method, using a long glass capillary tube, in which he is more or less successful in isolating bacteria in putrefying blood. Through the use of improved and simplified inoculation methods, such as the use of the cornea as inoculation site, our understanding of the growth of bacteria in animal bodies, particularly during septic processes, has been increased. Meanwhile, there remain many obstacles in our investigations of bacteria which must be overcome.

The greatest difficulties in studying bacteria microscopically are caused by their small size, the motility of these simplest of forms, and the fact that they do not possess strong light-refracting ability and are difficult to stain.

If bacteria were even smaller than reported to date, we might still be able to investigate them with our powerful immersion lenses; these can differentiate the linear systems in diatoms, which are much finer than the smallest bacteria. Primarily, what makes bacteria difficult to investigate is that they do not possess sharp outlines and are always found in vital or molecular motion.

It is impossible for one to measure an individual bacterium in a mass of them, or to fix them in a position from which a satisfactory sketch might be made. Tiny rods and cocci dance from the field and disappear in the thick mass of other bacteria, or they rise above or fall below the focusing plane. If the bacteria are united and immobilized in a zoogleic mass, they do not appear as a mass of related bodies; because of their light refraction, they give a cloud-like picture, single chains or groups hardly ever being seen.

In addition to hindrances due to the bacteria themselves, it appears to me that investigations have not been made on a method that would show the bacteria in their natural form and position (except when they are imbedded in animal tissues) and conserve and photograph them free of all distortions.

I need hardly emphasize the usefulness such a technique would have in the study of a collection of microscopic preparations; important discoveries could be communicated by sending preserved preparations to other microscopists for corrections of erroneous opinions and thus serve as a rapid means of verifying new findings.

One encounters many incomplete observations and false assertions concerning what bacteria ought or ought not do, and these would not be

published to swell the bacteriological literature to such a turbid stream if, for each object seen, the investigator had to submit preparations for demonstration.

If in our studies, a collection of microscopic preparations, for example, of *Spirochaeta plicatilis* and the spirochaete from tooth films, were easy to obtain and observe, this would be most helpful, since recently the existence of the former was in doubt and the latter was confused with the spirochaete of relapsing fever.

In other natural relationships, not necessarily concerning preservation of specimens, it would be of help to see drawings, but with bacteria this expedient is unfortunately only incompletely developed. At the outset it appears hard to believe that such simply constructed bodies cannot be sketched easily, yet it is true. Even with the largest bacteria, the extremely small size differences require such delicate and fine lines in drawing that the true reproduction of bacteria requires extraordinary care. It is questionable whether it is possible to draw the smallest forms accurately and in such a manner that they cannot be mistaken for similar forms. Most drawings are purely schematic and neglect the proportions to such an extent that it is impossible to compare them with the original. Many are so carelessly done that they are of little more use than if the author had not even seen the bacteria originally. Drawings of this sort can hardly serve as proof, however correct the observations, and in any event cannot be used for resolving a disputed point.

In order to obviate these difficulties, I have devised a method which, stated briefly, consists in drying bacteria-containing fluids in very thin layers on glass slides; with the bacteria thus fixed in a plane, they are stained with a dye and then washed to return them to their natural forms now made distinctly visible; the preparation is covered and sealed in a preserving fluid, and can then be used for making authentic drawings and photographs.

I shall now proceed to a detailed consideration of the various parts of this method.

Drying

The preparation of a thin, dry film of a fluid containing bacteria is very simple. After one has studied the fluid (for example, putrefying blood, tooth film, the upper layer of a fermenting infusion, and the like) and determined the form and motility of the bacteria in the usual manner, one removes a drop of the liquid with the tip of a scalpel and prepares a circular film on a cover slip about 0.5 cm. in diameter and as thin as possible. One then places this over a depression slide and locates the drop microscopically to ascertain whether the previously observed forms are present in large numbers. The more uniform the fluid is, the smaller the drop needed, and it is possible to

streak out the material on the slide. The material must always be prepared as a very thin film, so that the bacteria, blood corpuscles, etc., do not cover one another, but lie with some clear space between them. The thinner the film, the quicker it will dry. Usually the preparation is ready for further treatment after several minutes. Proteinaceous fluids, particularly blood, require somewhat longer, possibly an hour.

Slides thus prepared can be kept for weeks, and perhaps a month, if protected from dust, without alteration of the dried bacteria. This procedure has the advantage that as soon as this point is reached, if circumstances do not permit further work, the preparation can be set aside until later. I have devised a case for twenty slides, so arranged for storage of microscopic preparations that I can easily carry it, or certain sections of it, at any time to the sick bed or wherever there are opportunities for examining fluids for bacteria. Slides of dried bacteria are easy to mail. Thus I have, through the aid of F. Cohn, received slides of dried blood from cases of relapsing fever sent by F. Albrecht in Petersburg; he had prepared the slides for other blood tests, and the spirochaetes could be photographed.

An objection against drying may naturally be raised, that, like other microscopic material, the form of the bacteria must be altered to a considerable degree. I was certain that by washing (soaking) the preparations, the original form would be recovered. Soon after I began these studies, I saw, to my surprise, that bacteria did not, like most infusions, monads, and microscopic plants, disappear or shrink to an unrecognizable mass, but rather remained as rigid bodies surrounded by a slime layer, and thus they adhered to the glass without alterations in length or breadth. In fact, every bacterium possesses a slime layer, ordinarily invisible (which incidentally explains other properties--zooglea formation), but recognizable after drying; and each cell is surrounded by a sharply limited transparent margin. It is true that when fluids containing bacteria are dried, some are covered with a colloidal or crystalline mass and perhaps rendered indistinct; however, at the edges of a dried drop of fluid one finds isolated cells, which are eminently suited for demonstrating the stability of form of dried bacterial cells.

The single outstanding changes that occur are the flattening of cocci, lobation or ramification of zooglic masses, and the transformation of spiral forms to a wavy line. These inconveniences are easily avoided if one washes the preparation, in the manner to be described later, immediately after the last trace of visible moisture disappears from the cover glass. The slime layer of the bacteria swells fully, and the zooglic masses and spiral forms resume their natural forms. Proof of this is seen in the photographs of *Zoogloea ramigera* and *Spirillum undula,* in which this manner of treatment was used. Fine spirilla with small coils lose so little of their natural form on drying

that one can preserve and photograph them in the dry state. Examples are given in the photographs of spirochaetes from tooth film and the flagella of bacilli, taken after drying.* These photographs also serve as definite proof that drying alone is a definite aid for investigating bacteria. An oral *Spirochaeta,* which appears transparent in the saliva due to similarity of light refraction, appears distinctly after drying, and in fluids not stained, the difficultly observable flagella may be visible.

Later, in describing a photograph of the anthrax bacillus, I shall discuss the characteristic alterations it undergoes on drying.

Washing and Staining

The second step of the method consists in washing and staining the dried bacterial film.

One places the cover glass containing the dried bacterial film in distilled water or glycerin, and dips it up and down in the liquid. It is not necessary to use this fluid further in the preparation of films.

If one places the slide in absolute alcohol or, better yet, in a solution of chromic acid (0.5 per cent), the film becomes insoluble in water and glycerin, but an undesirable secondary reaction of this hardening is that the slime layer no longer swells and the bacteria adhere very tightly to the glass, or are imbedded in the ground substance, and their natural shape cannot be recovered. As a means of making the bacteria swell again without loosening them from the slide, I devised a solution of potassium acetate (1 part in 2 parts distilled water). In this solution, the bacteria again assume their original form, but they are also more transparent than previously. With the larger forms and with spore-forming bacilli, in which the spores are strongly refractive, they are seen distinctly. A further excellent quality of potassium acetate solution is that after the bacteria have resumed their original form, they do not change further. One may employ this as a preserving fluid and seal the films under it. Preparations 16 months old show no change, and apparently they will be useful for a long time.

In most cases, particularly when one is working with small forms, too transparent for accurate research and photography, the use of stains is effective in making them visible. I have investigated the various dyestuffs used in microscopy and in dye works, but the aniline dyes are best of all for staining bacteria.

Bacteria stain rapidly and completely with aniline dyes; in fact, one can use these dyes as reagents for differentiating bacteria from crystalline and amorphous precipitates, as well as from fat droplets

*Koch's photographs are not presented in this chapter. *Editor.*

and other small bodies. Moreover, the aniline dyes act in aqueous solutions in the same manner as does potassium acetate, in that the films may be washed in them without detaching the bacteria from the glass. I have used only aqueous solutions of aniline dyes, preferably methyl violet and fuchsin. The others, such as safranine yellow, eosin orange, methyl green, iodine green, and blue, do not dye so intensively, nor are they as stable. For single objects, fuchsin appears somewhat better, since it is not so intense as methyl violet. Usually the latter gives better results. Among the various brands of methyl violet, I have used the blue preferably (in price lists under aniline dyes it is described as methyl violet BBBBB).

Later it occurred to me, among several others, that bacteria could be made visible not only to the eye but to the photographic plate as well, and I again directed my attention to aniline dyes and their chemical relationships to light, noting the parts of the spectrum they would not transmit. In this regard I obtained best results with aniline brown, designated as New Brown.

The use of aniline dyes is simple, as shown in the following briefly described method.

I add one drop of a concentrated alcohol solution of methyl violet or fuchsin to 15 to 30 gm. of distilled water, so that it is colored intensely; then I add one drop from a small pipette to the film to be stained, and agitate the dye over the film for several seconds. Then I remove the dye, the film being more or less blue, and examine it to see whether it is stained sufficiently or not; and if not, I add more dye until the desired coloration is attained. After several trials one soon discovers the correct time for staining and the proper concentration of the aniline dye solution. If too weak, it will be dissolved from the film; if too strong, it will stain the background substance in which the bacteria are located too deeply, and they will not be sufficiently contrasted.

When the correct staining time has elapsed, I wash the dye from the film or absorb it completely with blotting paper; distilled water or a dilute solution of potassium acetate (1:10) may be used. Single preparations may be treated differently; some may be washed off with distilled water, others may not.

Staining with aniline brown differs somewhat from that described for methyl violet or fuchsin. Preparations stained with aniline brown lose their color if treated with potassium acetate, and therefore they must be preserved in glycerin; so after preparing them as outlined above, I cover the films with a drop of a concentrated solution of aniline brown in equal parts of water and glycerin (which must be filtered occasionally) and allow it to stand for one minute. The bacteria are then stained sufficiently, and the dye solution may be washed off with pure glycerin.

Proteinaceous matter, blood, pus, and the like, which stains poorly

with aqueous solutions of methyl violet or fuchsin, yields with aniline brown, and glycerin a wholly superior preparation, which serves especially well for photographic purposes.

Preservation

One may use Canada balsam, a concentrated solution of potassium acetate, or glycerin for preserving the stained preparations.

In preparations of this kind, swarms of vibrios, bacilli, and micrococcal clumps present an extraordinarily beautiful and graceful picture. Unfortunately, zoogleic masses and the larger bacilli appear flat. I have not yet succeeded in obtaining good photographs of Canada balsam preparations. I do find, on the other hand, that preparations in Canada balsam keep longer than those in other mediums, and this should make it quite useful for slide collections.

If methyl violet- or fuchsin-stained preparations are to be photographed, and one wishes to see them in their natural form, they must be covered immediately, after removing the dye from the film, with a potassium acetate solution (1:2) and sealed.

Glycerin may not be used for covering such preparations because it extracts the dye from the cells. Glycerin is the best preserving medium for aniline brown preparations.

Photographing

Photographing bacteria differs in no important way from photographing other microscopic materials. In all cases the bacteria are small transparent bodies, and not easily photographed. Yet, if prepared in the manner described above, they can be photographed under the cover slip, using the strongest possible oil-immersion system. As previously pointed out, strong light refracting ability, as brought about through staining the bacteria with aniline brown, represses transmission of the light chemically, and allows the photographic process to be accomplished.

Under satisfactory conditions, one may photograph living bacteria provided they are non-motile (as shown by photographs of the anthrax bacillus). Obviously in such photography there must not be too much light. The advantage of this method is that preparation of films and staining is not required. I do not doubt that all resting bacteria, particularly micrococci, can be photographed in the living state, and specific experiments regarding this will be presented later.

Spore-bearing bacilli and filaments, because of the light refracting ability of the spores, are best photographed unstained.

I must emphasize that I have not succeeded in obtaining absolutely sharp outlines of bacteria. In viewing photographs of diatoms, and being accustomed to the usual sharp outlines of bacteria in drawings,

I thought at the beginning of this work that something was wrong with the method. Having the strongest system of lenses available to me (Seibert's immersion system 8 and 9), I am convinced that, in reality, bacteria do not appear in sharp outline. I have therefore concluded that the bacterial cell is not sharply delimited and that the slime layer flows out of the latter by degrees.

I should like to emphasize that the photographic plate generally gives a better or more exact microscopic picture than the retina of the eye.

The light-sensitive plate is in reality an eye, which is not blinded by bright light, and does not grow weary of continuously differentiating small variations, or possess other defects to which vitreous humor exercises are subject. I have often found in a negative of a really sharp picture, a fine object, for example, a flagellum, which I could detect under the microscope only after extreme difficulty, and only under the most perfect illumination conditions.

Fine measurements of very small, transparent objects, which cannot be readily done with the microscope, can be readily made from a negative. Many disputed questions concerning fine structure can be easily resolved with the aid of a photograph, particularly if instead of the hitherto-used blue and red dyes, other yellow, brown, or red dyes are employed, dyes which because of their chemical constitution do not transmit all parts of the spectrum. Further investigations with the latter dyes will yield still better photographs of bacteria, and likely make it possible to photograph Canada balsam preparations.

There follows a technical description of Koch's camera and equipment, and a description of his photographs. Editor.

Further Readings

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VII.

JOSEPH LISTER

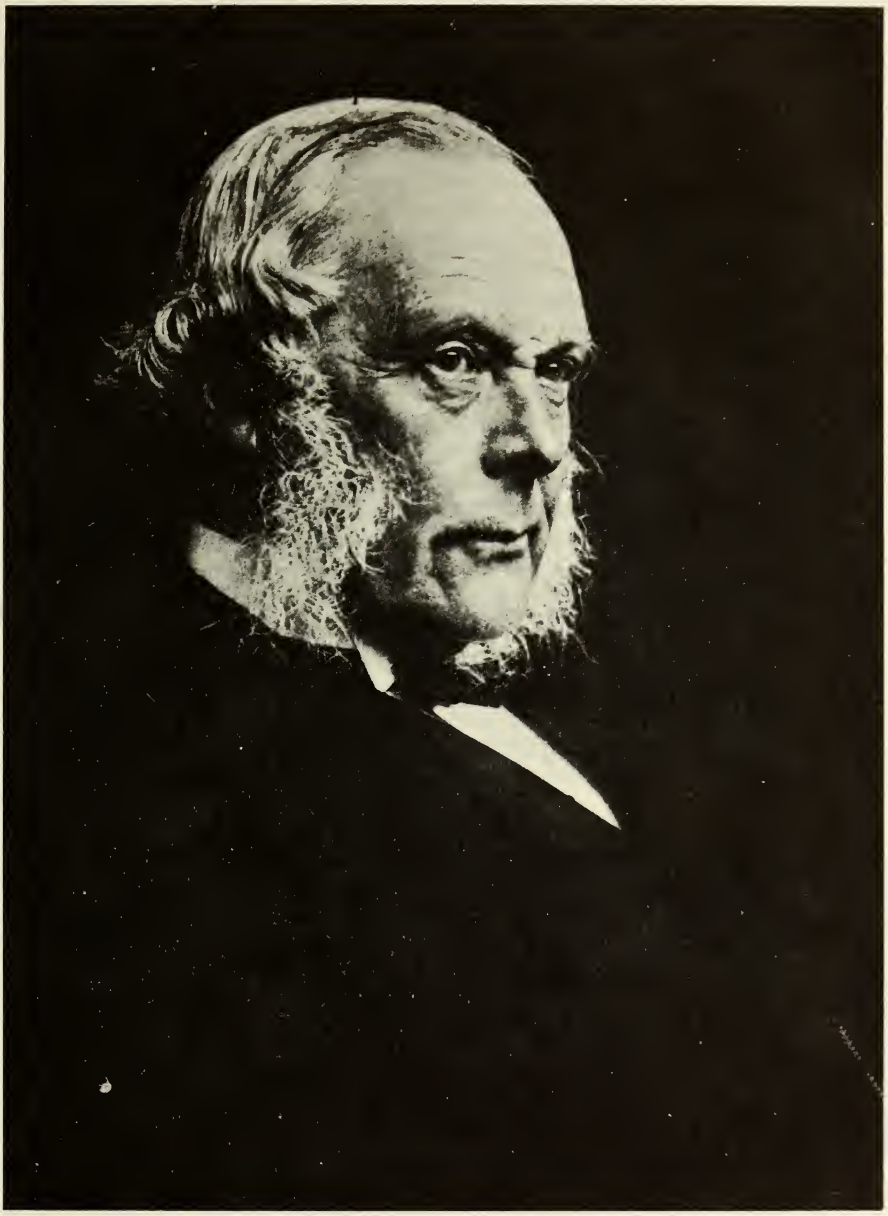
(1827 to 1912)

Joseph Lister was born in Upton, Essex, England, April 5th, 1827, and he died in Walmer, February 10th, 1912. He was the son of J. J. Lister, F.R.S., who made prominent contributions to optical science. Young Lister received the M.B. at University College, London, and became a fellow of the Royal College of Surgeons. In 1853 he went to Edinburgh, where he was house surgeon at the private hospital of James Syme. In 1860 he was appointed professor of surgery at Glasgow; but in 1869 he returned to Edinburgh, succeeding Syme as professor of clinical surgery. After 1877, Lister occupied the chair of surgery until his retirement in 1893.

Lister is well known for his part in developing antiseptic surgical methods, and for his contributions to bacteriology. In 1857 Lister had written a classic monograph entitled "An Essay on the Early Stages of Inflammation." Since the convincing experiments of Pasteur, in which the role of bacteria in putrefaction and fermentation was definitely proved, Lister worked towards preventing surgical infections. In 1865 he began using carbolic acid for this purpose. Initially, a piece of lint saturated with this compound was used; but later a carbolic acid spray was tried, and finally various kinds of surgical bandages were evolved. Lister chose carbolic acid when he learned that the city of Carlisle used it successfully to rid garbage of odor and various protozoa. Lister also discovered the organism which causes the "natural souring" of milk, *Streptococcus lactis* (1873).

In 1897 he was made Baron Lister; and in 1902 he received the Order of Merit. Lister was president of the Royal Society of London from 1895 to 1900, and president of the British Association for the Advancement of Science in 1896. He helped found the British Institute of Preventive Medicine in 1891, and was the recipient of numerous honorary degrees, prizes, and orders.

Lister was among the first to obtain a pure culture using a liquid medium. He did this by means of a specially constructed syringe



National Library of Medicine

Joseph Lister

equipped with a nut, *a*, and a finely threaded piston rod, *b* (Figure 9). The piston could be adjusted to deliver 1/100 of a minum (0.00062 ml.). Using such a sterile syringe and dilutions of sour milk, it was theoretically possible to transfer to boiled milk a quantity of fluid calculated to contain a single organism (Figure 1). Modern bacteriologists still use the technique of "enrichment" followed by dilution in their work on isolating pure cultures. In this manner, Lister discovered an organism he named *Bacterium lactis* (*Streptococcus lactis*), the cause of the natural souring of raw milk.

The following paper is an expansion of an address given before the Pathological Society on December 18th, 1877, and published in *Transactions of the Pathological Society,* XXIX (1878), 425-467. In this address Lister attempted to indicate the relationship between a specific microorganism and the fermentative change induced during its growth in a specific medium. The extension of such ideas to the surgeon's problem of putrefaction of wounds is obvious, but it must be noted that Lister wished to prove that microorganisms were the *cause* of fermentative changes and not the *result* of them. It would then follow that if bacteria could be prevented from contaminating surgical wounds and incisions, gangrene and pyemia, then so common, could be prevented.

ON THE LACTIC FERMENTATION, AND ITS BEARINGS ON PATHOLOGY[1]

A few years ago it would have seemed very improbable that the souring of milk should have any bearings upon human disease; but all will now be ready to admit that the study of fermentative changes deservedly occupies a prominent place in the minds of pathologists.

In order that any sure steps may be taken to elucidate the real nature of the various important diseases which may be presumed to be of a fermentative nature, such as the specific fevers or pyemia, the first essential, as it appears to me, is that we should have clear ideas, based upon positive knowledge, with regard to the more simple forms of fermentation, if I may so speak—more simple because they can be conducted and investigated in our laboratories.

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[1]This communication, made to the Society without manuscript, December 18th, 1877, is here given in its original form. In preparing it for the press, however, matter has been introduced, including some points into which the time at my disposal did not allow me to enter, and also some facts since ascertained, together with considerations arising from them.

It may be said, indeed, that such information has been already afforded us by the researches of Pasteur and others who have followed in his wake, tending to prove that all true fermentations of organic liquids are due to the development of organisms within them; and I confess that for my own part I am disposed to agree with that view. But this opinion is by no means universal in our profession. We meet with statements by men of very high position, both as physiologists and pathologists, to the effect that in various fermentations—such, for example, as putrefaction—the bacteria which are found to be present may, for aught we know, be mere accidental concomitants, not causes, of the fermentative change. And such being the case, it seemed desirable to obtain, if possible, entirely conclusive evidence upon the subject.

About four months ago I made an attempt of this kind with regard to the lactic fermentation; and I propose on the present occasion to bring forward the results arrived at, and at the same time to afford the members of the Pathological Society an opportunity of seeing with their own eyes samples of the preparations which resulted from that inquiry, and on which my conclusions are based.

First, however, I desire to describe my method of experimenting, which, in its present simplified form, has never been published. It is based, in the first instance, on the fact which experience has now amply demonstrated, that if we have a vessel like this liqueur glass, *A*, in a state of purity, covered with a pure glass cap, *B*, the capped liqueur glass being further covered with a glass shade, *C*, and standing as a matter of convenience on a plate of glass, *D*, any organic liquid contained in the liqueur glass, provided it be free from living organisms at the outset, will remain without any organic development occurring in it as long as the arrangement of the glasses is left undisturbed. (Figure 1).* Or, in other words, although an interchange is constantly taking place between the gases of the atmosphere and those in the liqueur glass—for the cap does not fit at all, and the shade is not air-tight—yet the double protection of the glass cap and the glass shade effectually prevents access of the atmospheric dust to the liquid; and if the dust is excluded, organisms do not occur in it.[2]

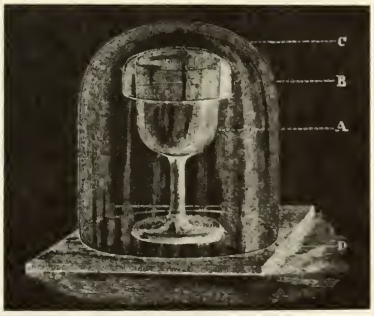

Figure 1

*Lister's woodcuts are reproduced by courtesy of the Royal Society of Medicine and J. M. Kutschbach. *Editor.*

[2]A curious exception to this general truth was met with by R. J. Godlee in the course of his experiments made by this method upon the vaccine virus, re-

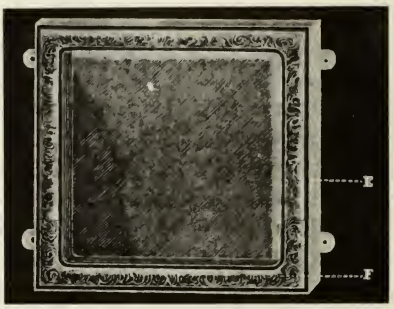

Figure 2

The glasses are obtained pure by means of heat. I find that exposure to a temperature of 300° F. for two hours is sufficient to deprive all living material of its vitality. But it is not enough that the glasses should be so heated; it is necessary that the air which enters them during cooling should be filtered of its dust. This I secure by heating them in a cast-iron box, the door of which, *E*, is here shown. This door has its circumferential part in the form of a groove, capable of being packed with a considerable mass of cotton wool, *F* (Figure 2). The door can be screwed by means of nuts (*G*, Figure 3) against the edge of the box (represented by dotted lines in the sketch); and the cotton wool, having the narrow rim of metal thus firmly pressed against it, serves as an effectual filter of the air that passes in during cooling. But then it is essential that the heat be so equably distributed as to avoid heating any portion of the cotton to such a degree as to destroy its physical properties. The cotton wool which you now see in the lid has been used for several experiments; yet you observe it is only slightly browned; it is not singed at any part, as it would have been unless the heat had been nearly uniform. This uniformity of the heat is provided for by having three shelves of sheet iron, (*H H H*), interposed between the large Bunsen's burner, *I*, and the bottom of the box, so as to prevent the heat from acting directly upon it; while at the same time the box is covered over with a cover of sheet iron, *KK*, which reaches nearly to the ground, and, while it checks radiation, compels the heated air to travel over the whole exterior of the box and escape by holes at the top of the cover, whence it is conducted into a chimney by the tube, L. By these two means combined, the shelves below and the

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corded in the Transactions of the Pathological Society, XXVIII. He found that *Penicillium glaucum* made its appearance in some of his glasses in a way that seemed unaccountable, till he discovered that the cupboard in which the vessels were kept was infested with acari, and that these creatures crawled up and into the glasses. He further found, on examining some of these acari under the microscope, that spores of the *Penicillium* were to be seen actually adhering to their hairs. The wonder now came to be, not that that fungus should have entered the glasses, but that other organisms, such as bacteria, should not have been also carried in. If there were any reason to apprehend the intrusion of such creatures, they might no doubt be easily excluded by placing each glass plate in an ordinary dinner plate containing glycerin, so as to surround the glass plate on all sides with the viscid liquid.

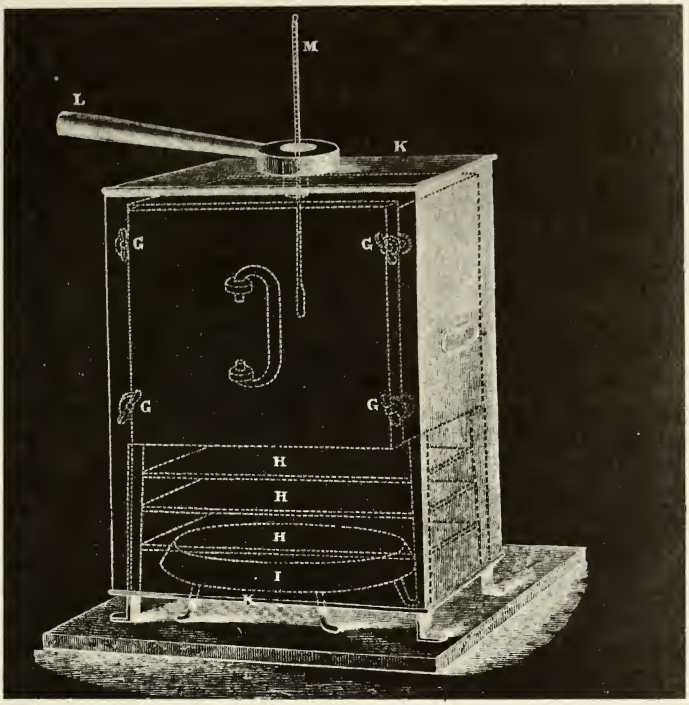

Figure 3

cover round about, we get the result which you see here. The cotton at the top of the box is browned to just the same degree as that at the bottom.

Into such a box (about 1 foot in its three dimensions and furnished with a shelf in the middle) we may put a dozen sets of covered glasses such as I have described, together with their glass plates and shades. An aperture in the top of the box, well packed with cotton wool, transmits the thermometer, *M*, to show when the temperature of 300° F. has been attained; and when this or any higher degree short of about 350° F. has been continued for two hours, the gas is turned off and cooling is allowed to take place; and when the apparatus is quite cool, the covered glasses may be removed with confidence that they are perfectly free from living organisms.

In the next place, how shall any pure organic liquid be introduced into one of these purified liqueur glasses without risk of contamination? This we are now able to manage in a comparatively simple

Figure 4

manner. The liquid is introduced by means of a flask of this form, *N*, having a bent spout, large at the commencement and comparatively narrow in its shorter terminal part, *O*, beyond the bend (Figure 4). The large size of the first part of the spout prevents it from ever acting like a syphon; and the result is that when liquid is poured from such a flask and the vessel is afterwards restored to the erect position, the end of the nozzle remains valved by a drop of the liquid; and this guards the orifice, so that regurgitation of air can never take place through the nozzle. And the mouth of the flask being covered with pure cotton wool, *P*, the air that enters the flask during the pouring out of the liquid is filtered of its dust by passing through the cotton. When the decantation is completed, a piece of rag wrung out of a strong watery solution of carbolic acid (1:20) is applied to the orifice of the nozzle, and, by capillary attraction, sucks out the drop; after which a cap of carbolized cotton wool (*Z* in Figure 6) is tied securely over the nozzle, the ligature obtaining a purchase upon the projection, *O*, upon the tube.[3] When this has been done, the liquid, if it was pure to start with, and the flask also pure, will remain ready to be used again in a pure condition a month or even a year later if required.

Now as to the means of preventing the entrance of dust into the liqueur glass while it is being thus charged. Suppose I were going to

[3]Cotton wool intended for a cap to cover the mouth or nozzle of a flask is conveniently applied between two pieces of open muslin, which ensure that the cotton shall remain as a uniform layer, and also permit it to be readily removed without leaving any of the cotton adhering to the glass. The best material for the ligature I have found to be very fine iron wire, tied firmly in a half knot, which is secured by giving the ends of the wire a bend, so as to convert them into hooks. A good mode of carbolizing the cotton wool is to treat it with a solution of 1 part of carbolic acid in 100 parts of anhydrous sulfuric ether. The cotton greedily imbibes the liquid into all its parts and the ether evaporating, leaves the carbolic acid behind in the cotton, which is thus rendered highly pungent and antiseptic.

Figure 5

charge this glass, *Q*, from this flask (the spout of which is alone given in Figure 5), containing Pasteur's solution introduced on August 7th, yet you observe, remaining as pure and clear as when it was first prepared, although it has served for charging various liqueur glasses. I should remove the bottom cap from the nozzle, and instantly slip the end of the nozzle into the opening which exists in the center of this half of a substantial India rubber ball, *R*, previously steeped in a strong watery solution of carbolic acid. The caoutchouc absorbs carbolic acid into its substance, so that, even though dry after such steeping, it is powerfully antiseptic. I then remove the shade and take off the glass cap, and immediately substitute the cap of India rubber on the nozzle of the flask (as shown in the sketch). Fluid is now poured in, the flexible caoutchouc acting as a hinge during the movements of the flask, while the antiseptic cap excludes all living organisms; and the instant the flask is withdrawn the glass cap is replaced and the shade put on. The hemispherical form of the cap also prevents lateral currents of air from depositing dust on the drop at the nozzle while it is being moved from one glass to another, and a second, a third, or a dozen glasses may be thus charged in succession; and experience shows that this mode of procedure is so secure that the liquid will remain uncontaminated in such a series of vessels until it dries up through atmospheric influence.

The last point is, How do we get the flask in a pure state with a pure liquid in it? The flask itself is purified together with its caps of cotton wool over the mouth and nozzle by being heated in the hot box. And here we have another great advantage arising from the uniform temperature of the box sufficient to purify cotton without impairing its physical properties. We have, therefore, a simple means of securing a pure flask to begin with.

Next, we wish to introduce into it a pure liquid. There is one organic fluid, peculiarly adapted for experiments on this subject, which can be got in the pure state with very little difficulty, and that is unboiled urine, provided we have a healthy urethra to deal with and a healthy bladder. All we have to do is to wash the surface of the glans penis thoroughly with a water solution of carbolic acid, 1 to 40, in which strength it may be applied to the lips of the meatus urinarius without causing inconvenient smarting, and having thus merely purified the external integument, remove the cotton cap from the orifice of the flask and at once apply it to the organ and direct the patient to

micturate. The purified glans takes the place of the caoutchouc cap, and as the urine enters no regurgitation of air is possible. As soon as the act of micturition is completed, a freshly carbolized cotton cap is tied over the mouth of the flask, and as surely as this process is properly performed, will you have the unboiled urine, together with its vesical mucus (which used to be regarded as the special ferment of urine), remain for any length of time free from development of bacteria or any other organism, and that whether the urine be acid or neutral in reaction.[4]

But suppose we have to deal with a liquid contaminated with organisms, like milk obtained from a dairy, we must purify it by heat. For this purpose I have always found exposure for an hour to a temperature of about 210° F. sufficient.[5]

I say 210°, not 212° F., which is the boiling point of water, because the way in which we have proceeded is, after introducing the liquid into the purified flask, to immerse the vessel, to a higher level than that of the contained liquid, in boiling water; and, in consequence of a certain degree of evaporation which takes place through the cotton caps which cover the mouth and nozzle of the flask, the temperature of the liquid is prevented from ever rising fully to the boiling point. We thus avoid the occurrence of ebullition; and this is a very great advantage, as we get rid of frothing; and, as already stated, the temperature which is attained proves adequate for the destruction of

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[4]For further details on this subject, see *Transactions of the Royal Society of Edinburgh,''* XXVII (1875), 315 *et seq.*

[5]In making this statement I proceeded upon a pretty extensive experience with various liquids, such as water, Pasteur's solution, and turnip infusion, as well as milk. In my experiments with the last-named liquid, I had obtained it fresh from the cow milked directly into a glass vessel purified with carbolic lotion and then rinsed with water and having filtered it through a fine cloth, exposed it to heat with as little delay as possible. But between the time of the meeting of the Society and that of going to press, I have found that milk which has been kept for some hours cannot be purified so easily. This I am inclined to attribute to the fact that milk, unlike the other liquids referred to, is a heterogeneous fluid containing oily particles in suspension, which tend as time passes to aggregate and coalesce. And it is quite conceivable that bacteria enclosed in such oily masses may be protected by them from the action of the hot watery liquid, the drops of butter acting as small portions of cheese have been found to do by some other observers. For it is a well established fact that bacteria withstand a much higher temperature in the dry state than when acted on directly by hot water. I have also seen reason to believe that large numbers of bacteria grouped in masses are more resisting to the action of heat than isolated bacteria, as if the circumferential members of such a mass protected the central ones, and this circumference may perhaps aid in explaining the point in question. Again, if we consider the adventitious particles introduced in the form of dirt from the milkman's hands, etc., we cannot be surprised that difficulty should be experienced even with fresh milk, unless special precautions are taken to promote cleanliness.

organisms in the liquid. All we have to do then, supposing the interior of the flask perfectly pure, is to introduce the liquid into the lower part of the flask with a view to immersion in a saucepan of boiling water. But then the most scrupulous care must be taken that no portion of the liquid so introduced shall come into contact with the upper parts of the interior of the flask; for any particles deposited there would fail to be acted on by the full heat of the water in the saucepan.

Now the mode in which I filled the flasks in my earlier experiments was this. Having provided myself with a rag soaked with a strong solution of carbolic acid (1:20), I washed with it the exterior of a long funnel, and, wrapping the rag round the lower end of the funnel in sufficient mass to cover the mouth of the flask, I substituted the mass of antiseptic material for the cotton cap with which the mouth was previously covered, pushed down the funnel through the rag, poured in the liquid, and withdrew the funnel, taking good care that the drop at its extremity did not touch the interior of the flask. A freshly prepared carbolized cotton cap was then applied to the mouth of the flask at the moment of withdrawal of the antiseptic rag, and the flask was immersed in the saucepan.[6]

If I proceeded in this way with Pasteur's solution or with turnip infusion, I always had success; but when I did the same thing with milk, time after time, to my great disappointment, I failed altogether. What was the explanation of the failure? Some persons might have said, "Oh! the explanation is very easy to find. There are in milk, complex organic molecules which, though as yet mere chemical substances, are, we imagine, ready to develop into living beings, and it is this complex constitution of the milk that makes you fail; whereas your Pasteur's solution is a comparatively simple material, and turnip infusion may, for aught you know, have its molecules more simply constituted than milk." I felt sure that this was not the true explanation, but that there must have been some defect in my method of procedure. It may perhaps have occurred to some of you what that defect was: It was this, that if we pour any liquid through a funnel, we invariably have air pass along with it. Air bubbles consequently formed upon the surface of the liquid, and those bubbles bursting, dispersed their dust in the air within the flask; so that it might well happen that

[6] Means must be taken to prevent the flask from being floated up by the water in which it is immersed. This I have done by tying the flask into a vessel, such as a soap dish, containing shot. The flask so weighted is placed in the empty saucepan, warm water is poured into a level considerably above that of the liquid in the flask, and brought to boil as quickly as possible and and kept simmering for an hour. A piece of thin Mackintosh cloth, cut so as to adapt itself to the flask and cover the top of the saucepan, has the double advantage of preventing the water from boiling quickly away and avoiding moistening of the cotton about the flask by drops of condensed vapor.

such particles, including perhaps some atmospheric organisms, might be deposited upon the upper part of the vessel, and so fail to be destroyed when the liquid was heated. But why should success be more likely with Pasteur's solution than with milk? Simply for this reason, that milk is a material which serves as a pabulum for almost all organisms. I once met with a bacterium, but only once, that would not live in milk; for extremely numerous as the varieties of bacteria appear to be, almost all of them seem to thrive in that liquid, whereas it is a common thing to find bacteria which, if put alive in Pasteur's solution, will not grow in it at all.

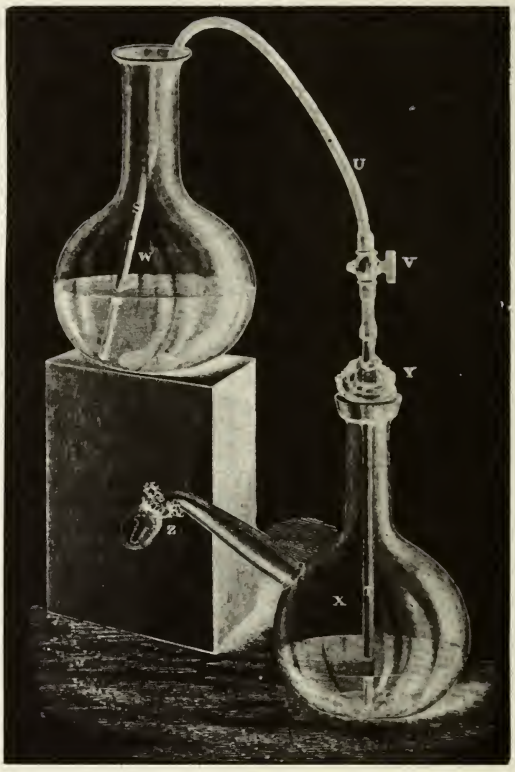

Figure 6

The defect in our method having been discovered, there was no great difficulty in correcting it. It was done by substituting for the funnel this syphon (see Figure 6), consisting of two glass tubes *S* and *T* connected by a tube of India rubber, *U*, with a stopcock, *V*, in the course of the caoutchouc tubing. The syphon is first completely filled with water, the temperature of which should be higher than that of the air, so that there may be no dissolved air given off to form bubbles. Then suppose this, *W*, to be the fluid that we wish to introduce into the flask, *X*. We pass one leg of the syphon into it; then turn the tap and permit a sufficient amount of fluid to flow out to ensure that all the water has escaped from the syphon; then turn off the stopcock and proceed in the manner already described for the funnel. The tube, *T*, having been washed with strong carbolic lotion, carbolized rag is wrapped round its lower extremity, and this is applied to the mouth of the flask as the cotton cap is removed; the tube, *T*, is pushed steadily down to the bottom of the flask through the

carbolized rag, *Y*, the stopcock is turned on, and the liquid passes into the flask without the smallest air bubble accompanying it. When a sufficient quantity has been introduced, the tap is again turned off, and the syphon is withdrawn through the antiseptic rag, and a fresh cap of carbolized cotton is tied over the mouth of the flask when the rag is withdrawn.

I have said that before I saw my mistake in using the funnel, I never succeeded with milk. Since I have adopted the syphon, I have charged many flasks and never failed. Here is a flask of boiled milk (or rather of milk that has been exposed to a temperature of 210° F.) prepared on August 7th, and remaining we may safely say as pure as it was then. You observe it is still perfectly liquid and unaltered in appearance. Now I venture to remark that this failure and correction of the failure are extremely instructive, as showing how the development of organisms, under circumstances in which we cannot at first explain their occurrence, may be really due to fault on our part, defect in our own manipulation.

So much for our method of procedure. I will now go on with the main subject of this communication. I selected the lactic fermentation as one peculiarly favorable for investigation: first, because the effects which it produces in milk are extremely striking and readily recognized—the solidification which takes place being obvious at a glance, and the souring as shown by test paper being also a very conspicuous change; and in the second place because the ferment which occasions these alterations is, in ordinary localities, a very rare ferment; and if it be rare, it is not likely that any defects in our manipulations will lead to its accidental introduction.

It may seem strange that the ferment that leads to the souring of milk should be rare, but such is the fact; in dairies it appears to be universal, but in the world at large it is scarce. If you charge a series of pure liqueur glasses with boiled milk and take off their caps so as to expose them to the air for about half an hour each, doing this for the various glasses at different times of the day or in different rooms, you will be sure to have organisms develop in all of them, of the nature of filamentous fungi and bacteria; and you will see fermentative changes ensue; but, so far as my experience goes, you will not see the coagulation and souring of the lactic fermentation, nor will you find under the microscope the peculiar organism to which I have given the name of *Bacterium lactis* (*Streptococcus lactis*), which is represented here (Figure 7) as it occurs in ordinary curdled milk, and which may be seen under one of the microscopes on the table, in milk taken from the cow yesterday.

This organism is a motionless bacterium, that is to say, exhibiting no movement except a slight jogging, occurring most commonly in pairs, but frequently in chains of three, four, or more individuals, each segment being of somewhat rounded form, more or less oval,

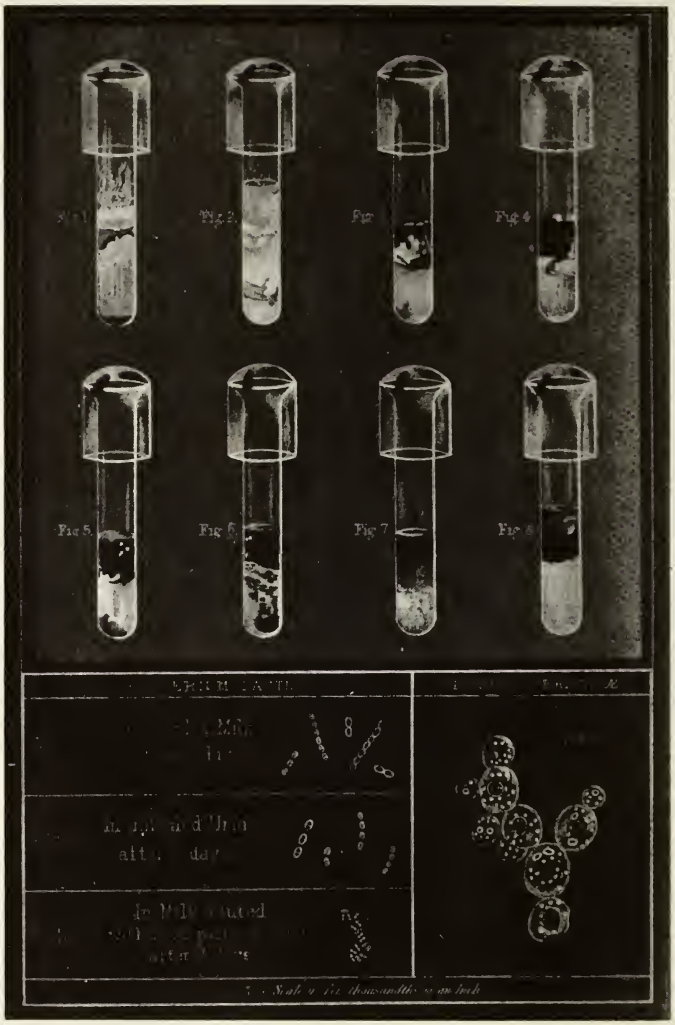

Figure 7. The text facing the plate as it was published in the *Transactions of the Pathological Society* reads as follows:

"The figures in this Plate illustrate Mr. Lister's paper on the Lactic Fermentation in its Relations to Pathology."

"Figs. 1 to 8 represent of natural size certain glasses of unboiled milk described in the text. They are examples of the alterations in appearance produced in milk by various different organisms other than the *Bacterium lactis*. From sketches taken October 1st, 1877."

"Figs. 9 to 12, Camera Lucida sketches, by Mr. Lister, of *Bacterium lactis* and *Torula cerevisiae* on the same scale, viz. magnified 1,125 diameters."

with the long diameter in the direction of the length of the chain, and often showing, on careful focusing, a line across their central part indicating transverse segmentation. They vary in diameter, as I shall have occasion to notice again, full-sized specimens measuring about $\frac{1}{20,000}$ in.[7] You always find them, as far as I know, in souring milk from a dairy, and a touch with the point of a needle which has been dipped into such souring milk will induce their development with great rapidity in a glass of boiled milk, together with the characteristic curdling and souring, showing that the boiled milk is thoroughly disposed to the lactic fermentation as soon as the appropriate ferment enters, while that ferment, as already stated, is not likely to occur as the result of exposure to the air of your study or any ordinary situation.

These little glasses (represented on a reduced scale in Figure 8) illustrate the same point as regards unboiled milk. Each is a small test tube provided with a cap made of the bottom of a larger test tube, and arranged along with five others in a stand made of pieces of glass tubing and stout silver wire, and placed under a common glass shade on a plate of glass. Four such sets of glasses, making twenty-four glasses in all, were purified by heat in the hot box, together with their plates and shades. Another larger glass, provided with a glass cap,

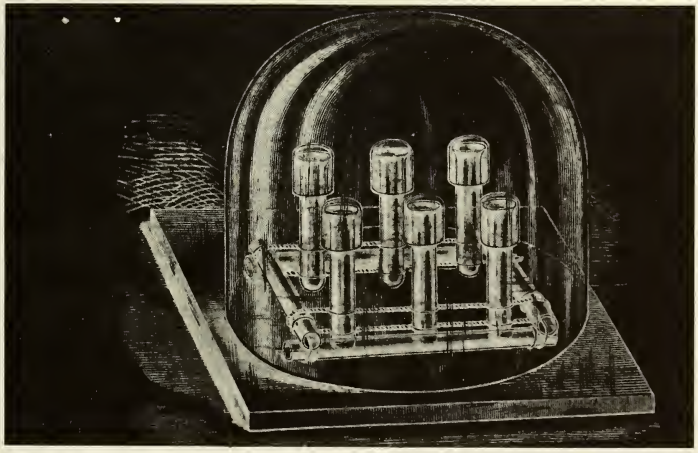

Figure 8

[7] In giving these as the characters of the *Bacterium lactis*, I do not wish to be understood as stating that this species can always be certainly recognized by its morphological features alone.

having been also purified by heat in the box, milk was drawn directly into it from a cow standing in a little orchard belonging to a dairy farm and within two yards of the dairy itself, the glass cap being removed immediately before the milking commenced, and re-applied as soon as the vessel was charged, the quantity amounting to about 2 oz. Then by means of a syringe connected with a purified pipette the milk was at once transferred in a room in the farm house, to the little glasses, each cap being replaced as soon as the glass was charged. Yet in spite of the care that was taken, organisms must have entered from the air of the orchard or of the apartment, possibly also from the cow's teat (though this had been previously washed by drawing milk from it just before the glass was charged); for you observe every one of the twenty-four glasses shows manifest evidence of alteration, and there can be no doubt that these changes are due to the development of organisms. No fewer than seven of them are seen, by aid of a pocket lens, to contain filamentous fungi of various sorts, for aught I know undescribed, and certainly none of those commonly met with in milk.[8]

Two of the glasses containing filamentous fungi are represented of natural size in the sketches reproduced in *1* and *2*, Figure 7. The other glasses present appearances which members of the Society, if they will examine them, will find to be exceedingly peculiar. No two are alike; some have a golden-yellow aspect (*8*), some a green tint (*7*), while many are studded with brilliant red spots (*3, 4, 5, 6*) either alone or associated with other colors, and the lower parts of the contents of the several glasses are changed to various degrees of translucency and shades of tint, implying different fermentative changes. But there is no reason to suspect in any of them the occurrence of the *Bacterium lactis*, or the lactic fermentation.

I have not had opportunity to examine the contents of these glasses microscopically, but in another experiment made in the same way a few days previously with twelve similar glasses, I found, just as here, some affected with filamentous fungi, and the majority altered in various ways in color, including definite specks of different shades of orange growing gradually, as the red ones in this set did, from a mere speck to larger and larger spots; and, examining with the microscope the contents of every one of those glasses, I found the orange specks to be composed of organisms in the form of little spherical granules peculiarly grouped, which I have elsewhere described under the name of *Granuligera;*[9] and other glasses, having alterations without such

[8] The filamentous fungi most frequently found in milk that is kept for a considerable period are the *Oidium lactis* [*Geotrichum candidum*], the common blue mold *Penicillium glaucum*, the common green mold of cheese, *Aspergillus glaucus*, and two forms of *Mucor*, the *M. mucedo* and *M. racemosus*.

[9] See *Transactions of the Royal Society of Edinburgh*, XXVII (1875), 319, and Plate.

bright and definite colors, presented bacteria of different appearances, but in no case did I find the *Bacterium lactis*.

I also performed other experiments of a very simple character which illustrate equally well the rarity of the lactic ferment in the air, even though it be the air of a cow house. The teat of the cow and the milkmaid's hands having been washed with strong watery solution of carbolic acid, milk was received directly into a purified flask of the kind above described, and from this a dozen purified liqueur glasses were charged in my study within about half an hour. All these glasses underwent fermentations, and exhibited organisms under the microscope, but none showed the lactic fermentation or the *Bacterium lactis*.

With regard to the cause of the strange, perhaps unprecedented, appearances presented by the milk in the little glasses shown to the Society, the explanation undoubtedly is, not that organisms of a specially rare kind were here present, but that species, perhaps exceedingly frequent in other media, had an exceptional opportunity for coming forward in milk, in consequence of the exclusion of ferments such as the lactic, the butyric, etc., which, when present in milk, as they commonly are, take the precedence of others, and so alter the fluid as to render it an unsuitable pabulum for the multitude of other kinds.

Here I have another set of little test tube glasses, twelve in number, with which the same experiment was repeated two days later, only with still more careful precautions against the entrance of organisms from the air or from the teat; but even here, although the appearances are less conspicuous than those in the other twenty-four, a careful inspection shows that, in ten out of the twelve, organisms of one kind or another have entered, but two of them remained perfectly unchanged in aspect six weeks after the performance of the experiment, and on examining the milk from one of these, I found it fluid, perfectly natural in reaction and in taste, and free from any organisms that could be discovered by the microscope. Thus was at length attained the object of these experiments with the little glasses, viz., the proof that unboiled milk, as coming from a healthy cow, like urine from a healthy bladder, really contains no material capable of giving rise to any fermentative change or to the development of any kind of organism which we have the means of discovering.

The lactic ferment is as scarce in ordinary water as we have seen it to be in the air. If I prepare a series of glasses of boiled milk in the way that has been described, and add to each a drop, say half a minim, of tap water, I find that fermentative changes, such as putrefactive or butyric alterations, occur in all, but that none exhibit the souring or curdling of dairy milk. An extremely instructive experiment is to inoculate each of a series, say ten, of such glasses with a very small drop of a constant size for all, which can be readily done by means of a syringe like this (Figure 9), having a graduated nut, *a*, revolving on a fine screw on the piston rod, *b*, each degree on the nut

corresponding to $\frac{1}{100}$ minim, so that either $\frac{1}{100}$, or $\frac{1}{50}$, or $\frac{1}{200}$, if it be desired, can be expelled at pleasure.[10] Supposing $\frac{1}{100}$ minim of water to be thus introduced into each glass, the result will probably be that fermentative changes of totally different kinds will occur in the various glasses, as indicated by striking differences of color in the contained milk and differences of odor in the air in the glass shades; and, what is extremely interesting, some glasses will escape all change.

This last fact proves at once the important truth that the fermentative agency in water, the existence of which was pointed out long ago by Burdon Sanderson, is not matter dissolved in the water, but consists of insoluble particles of some kind or other suspended in it. For if the ferment were dissolved in the water, every equal-sized drop would produce an equal effect. But the fact is, as already stated, that when drops of such minuteness are employed, some of the inoculated glasses escape altogether, and those which are affected exhibit different kinds of fermentation; and we thus learn from this simple experiment not only the truth that the fermentative material is in the form of insoluble particles, but that every minim of ordinary water contains several different kinds of ferments, which, though generally confused through being mixed up together, declare their individual peculiarities when isolated by this method of separation.

These various fermentative changes were invariably found associated with bacteria, those in different glasses sometimes showing themselves conspicuously different under the microscope, though often

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[10]It is essential to the precise working of this apparatus that there be nothing elastic in its composition; otherwise drops of varying size will be furnished by it. Thus, an India rubber junction between the syringe and the nozzle is inadmissible. The nozzle of glass tube, *c* is screwed upon the body of the syringe by means of the brass adapter, *d*, to which the glass tube is secured with a cement which I have found very useful on account of its power of resistance to the action of boiling water, viz. finely powdered gum copal brought to a liquid state by a high temperature and then applied to the objects to be cemented, which have previously been heated to an equally high degree. The presence of an air bubble in the water within the syringe would also by its elasticity vitiate accuracy of performance. This is got rid of, so far as the body of the syringe is concerned, by first accurately charging it with water that has been boiled to dispel its dissolved air. The water of inoculation, with which the glass nozzle is to be filled, is also raised to a somewhat higher temperature than the air of the apartment by placing the vessel containing it before the fire for a few minutes. The nozzle with its adapter is purified by boiling it in water in a saucepan for about half an hour, after which its fine end is immersed in the water for inoculation, and when it is filled beyond the bend, the other end is depressed, so that the rest of the filling may be done by syphon action. When a drop of water projects from the adapter, it is applied to a correspondingly projecting drop of water at the mouth of the syringe, and the two drops coalescing prevent the introduction of a particle of air when the nozzle is screwed on. With these precautions drops of exactly the same size can be ensured.

only distinguished by their effects. Thus, in one case the cream being particularly thick, a bright vermilion-colored point appeared after some days at the spot of inoculation, and gradually spread over the surface; and on examining with the microscope a portion taken with a purified needle from the vermilion spot, I found it teeming with bacteria, while a portion of the uncolored cream in the vicinity showed nothing but the usual milk globules. Here then was a remarkable change going on in the milk *pari passu* with the development of bacteria, and doubtless occasioned by them, yet the bacteria themselves were of ordinary double rod form and of about medium size.[11] But various as were the changes produced in the milk by inoculation with water, there was no instance in the course of several such experiments of the lactic fermentation resulting from it.

Figure 9

We see, therefore, from the facts which I have adduced, that the souring of milk, instead of being—as might naturally be supposed *a priori* from seeing it occur constantly in all milk brought from a dairy—an inherent property of the liquid, is a change which, whether in boiled milk or unboiled, requires the introduction of something from without, and that something a scarce article, both in air and in water, except in dairies. Indeed, even in a dairy, though it exists in all the milk in the pans, it does not necessarily follow that it is the most frequent ferment in the air.

I once took a glass of pure boiled milk to a dairy, and putting it down near one of the pans, removed its glass shade and glass cap, and left it exposed for a quarter of an hour, thinking it probable that the lactic fermentation would result. But it so happened that this was not the case. As a consequence of this exposure, a filamentous fungus made its appearance in the milk, and also a bacterium, but a bacterium associated with a most extraordinary

[11]These experiments were performed in the early part of the year 1875, and were briefly alluded to in a paper in the *Lancet* of that year. See *Lancet* (April 3, 1875), 470.

alteration, viz., viscidity in an extreme degree, reminding me of that of the glutinous drops that bead the spider's web. On introducing a needle into the top of the liquid, and raising it, I drew out a barely visible thread, which was a yard and two inches long before it broke. Such was the special fermentation that resulted from exposure to the air on that occasion even in a dairy; it so happened that if any particles of the lactic ferment were floating in the air at all, none fell into the glass.

This particular fermentation, therefore, from the conspicuousness of its effects and the rarity of the ferment under ordinary circumstances, seemed a peculiarly favorable one for investigation.

And now, before proceeding further, I desire to correct a mistake into which I fell when investigating this same fermentation some years ago; for, next to the promulgation of new truth, the best thing, I conceive, that a man can do, is the recantation of published error. In the year 1873, I gave, in the *Microscopical Journal,* an account of the behavior, as I supposed, of the *Bacterium lactis* in different liquids.[12] I stated that having obtained souring milk from a dairy, I inoculated a glass of uncontaminated unboiled urine with a small drop, and the result was the development in that liquid of organisms with a very different appearance from the bacteria which I had seen in the souring milk. The latter had the characters of *Bacterium lactis,* as already described (Figure 7, *9*), viz., pairs of chains of small oval bodies, with lines of transverse segmentation. Those in the urine, on the other hand, were broad and extremely long, often coiled up like a spirillum, though motionless, like the *Bacterium lactis.* There were, however, what certainly looked like transitional forms between the two.

From this urine I inoculated another glass of the same liquid, the result being a reproduction of the same large spirillum-like organism. I next inoculated from the second urine glass one of Pasteur's solution; and now obtained appearances different from any before seen, viz., instead of either the motionless chains of *Bacterium lactis* of the milk, or the spirillum of the urine, an actively moving, minute double bacterium. But on introducing a small drop of the Pasteur's solution so peopled into a third urine glass, I got back large coiled organisms resembling those of the former glasses of the same fluid, except that these exhibited languid motion. Yet the introduction of a small drop from this third urine glass into one of pure boiled milk was followed by souring and curdling, just as if the inoculation had been made with sour milk from the dairy. The apparently transitional forms in the first urine glass made me suppose that the spirillum was the *Bacterium lactis,* modified by its new habitat. I also examined

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[12]See *Quarterly Journal of Microscopical Science* (October, 1873).

in a cultivating glass, under the microscope, a drop of a mixture of fresh urine with a small quantity of the Pasteur's solution containing the moving bacteria, and found that the minute active organisms, first seen, gave place to larger, languidly moving specimens, and thus I thought I had evidence of a transition from one to the other. And lastly the efficiency of the contents of the third urine glass as a lactic ferment, in spite of the various forms assumed in the various media, confirmed my belief that I was observing one and the same organism in all the glasses. And if this was really the case, considering how thoroughly the organism must have been washed in the various media of all chemical material that might be supposed to be originally associated with it in the milk—particularly considering that the lactic fermentation does not occur at all in either urine or Pasteur's solution—the chain of facts appeared a strong confirmation of the view that this particular bacterium was really the cause of the lactic fermentation.

I was mentioning these facts a few months ago to an eminent physiologist, who took the view that after all bacteria might be mere accidental concomitants of the fermentative changes; and when I had finished my story, he said, "Well, I am convinced." I thought to myself, "If I have convinced this eminent teacher by these facts, they are worth proving more rigorously; I shall have a little time between giving up my surgeoncy in Edinburgh and going to London, and I will devote it to repeating the experiments; but this time I will make continuous observations under the microscope, and trace, if I can, the actual process of transformation of the organism from one form to another." Accordingly, I got some souring milk from the same dairy as before, and proceeded, in the first place, to inoculate from it a glass of uncontaminated unboiled urine, and also a glass of pure Pasteur's solution. The result in the case of the urine was that, instead of getting a large motionless spirillum, as in the corresponding experiment of four years previously, I found an active double bacterium of moderate dimensions. In the Pasteur's solution, on the other hand, instead of the minute, very actively moving bacterium of my former experience, I obtained only motionless bacteria of various dimensions.

Here then were the facts all wrong. What was the explanation? I had obviously got some accidental contamination of the *Bacterium lactis* with other forms, although in doing the inoculations I had introduced only a very minute portion with the point of a heated needle. So I determined to try if possible to get rid of concomitant bacteria of other kinds, and the way which occurred to me as a possible mode of doing this was to dilute the souring milk with so large a quantity of boiled and therefore pure water as to have on the average, so far as it could be estimated, only one bacterium of any kind to every one of the drops with which a set of glasses of boiled milk should be inoculated. If this could really be done, as the *Bacterium lactis* was certainly in

much larger numbers than any other kind, I might hope that some at least of the drops of inoculation might contain it isolated from other species, and that thus I might have the *Bacterium lactis* develop pure and unmixed in the inoculated milk. Accordingly, having obtained some souring milk from the dairy, I inoculated a glass of boiled milk from it by dipping the point of a heated needle successively in the two liquids, and when the odor of souring milk was perceptible in the air under the glass shade, I found bacteria present in microscopic examination, and endeavored to estimate their numbers in proportion to the liquid.

This was done in the following manner. By means of the syringe already described one or more hundredths of a minim could be measured with precise accuracy; and I found that $\frac{1}{50}$ minim exactly occupied a circular plate of thin covering glass, half an inch in diameter, so that when such a drop was placed on a glass slide, and a covering glass of the size mentioned and quite flat was put down upon it, all air was expelled from under the latter, and the rim of fluid that formed round about its margin was so narrow as not to measure a quarter of the diameter of the field of the microscope even when the highest magnifying power was used. In other words $\frac{1}{50}$ minim was disposed in a thin, uniform layer of the exact size of the covering glass. Hence the number of bacteria under the glass slip, that is to say in $\frac{1}{50}$ minim, was equal to the number of the bacteria in a field of the microscope multiplied by the number of times the area of that field went into the area of the covering glass. The micrometer gave the diameter of the field in thousandths of an inch; and the covering glass measured 500/1,000 in. across; and the areas of the circles were of course proportioned to the squares of those diameters. All that was needful therefore, in order to enable me to calculate the number of bacteria in $\frac{1}{50}$ minim, was to form a fair estimate of the number of bacteria per field, and this was done by counting the organisms in a considerable number of fields and taking the average.[13]

It so happened that two kinds of bacteria were seen under the microscope, one motionless, with the characters of *Bacterium lactis,* the

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[13] I found great advantage from placing the circular covering glass (with the liquid) on the under surface of a plate of thin glass, large enough to cover a chamber excavated in a thick glass plate. A drop of water sufficed to fix the edges of the thin plate of glass down upon the thick, and served also to prevent any evaporation taking place from the liquid to be examined into the air chamber, so that the liquid was maintained of constant quantity. At the same time, the object being on the under side of the thin plate of glass, an immersion lens could be used for examining it to its extreme limits without the inconvenience of the liability of the drop of water under the object glass mingling with the liquid examined, as would have been the case had the object been on the upper side of the glass plate.

other much less numerous, with longer segments and in active movement. As a rule, on examining milk which is undergoing the lactic fermentation but is still fluid, the *Bacterium lactis* is alone discoverable; but in this instance we had ocular proof of the admixture of another sort, though in much smaller numbers. As the result of the estimate which I made of the number of bacteria present in every $\frac{1}{50}$ minim, I found it necessary to dilute the milk with no less than a million parts of boiled water in order that every $\frac{1}{100}$ minim should contain on the average a single bacterium.[14] This having been done, $\frac{1}{100}$ minum of the infected water was added by means of the small syringe[15] to each of five glasses of pure boiled milk. The result of this inoculation was that one only of the five glasses was affected at all. The contents of four remained permanently fluid and unchanged, and when examined with the microscope after the lapse of 13 days, showed no bacteria of any kind. The milk of the fifth glass, however, was, in the course of the third day after inoculation, converted into a solid mass, and on examination was found to be sharply acid; and under the microscope there were seen among the granular masses of casein countless motionless bacteria with the ordinary characters of *Bacterium lactis*, specimens of which were sketched and are reproduced in this diagram (Figure 7, *9*). No other bacteria, however, were to be discovered; we had got rid of the moving species which was seen to be associated with it in the milk before dilution, and *a fortiori* it might be believed that other species, doubtless present in the original milk, but in too small numbers to be detected, had been avoided.

Having therefore presumably obtained the *Bacterium lactis* pure and unmixed, I proceeded to ascertain its behavior in other media. I inoculated a glass of uncontaminated, unboiled urine with it; and now, instead of either the moving bacterium which resulted from a

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[14]The dilution with this enormous proportion of water was readily done by dividing the process into two stages. First, by means of the small syringe with purified nozzle, $\frac{1}{100}$ minim of the milk was added to 200 minims of boiled water, which had been measured into a purified glass by means of a pure graduated pipette connected with a syringe, the end of the pipette adapted to the syringe having been stuffed with cotton wool before purification in the hot box, so as to filter air that passed into the pipette from the syringe. When the milk had become thoroughly diffused through the water, as indicated by uniform opalescence (the diffusion having been promoted by stirring radpily with a pure glass rod), a minim of this first dilution was transferred by means of another and smaller pure syringe-pipette into a second purified glass, which had been charged with 50 minims of boiled water. I need hardly add that the utmost care was used to avoid more than momentary exposure of the liquids manipulated, and of the purified pipettes or other apparatus employed.

[15]The nozzle of this syringe was purified by boiling it in water in the interval between its employment referred to in the above note and the inoculation of the milk glasses.

corresponding inoculation with sour milk a few days before, or the large coiled organism of the experiments done four years previously, there appeared a motionless bacterium with identically similar characters to those of the *Bacterium lactis* in the glass of milk, as will be seen from this diagram (Figure 7, *10*) made from a sketch taken two days after inoculation of the urine. Had either of the forms seen in the previous experiments been present, the insignificant motionless *Bacterium lactis* would probably have escaped notice altogether. In the course of the first 24 hours its growth was indicated by delicate white vertical streaks on the glass, without any turbidity of the liquid. Next day, however, the fluid was decidedly nebulous; but after that period the growth was exceedingly slow, and very little effect was produced upon the urine as regards either odor or reaction. Nevertheless, it still retained its power of acting as a lactic ferment when introduced into milk even after four days' residence in the urine; for having inoculated at this period eight glasses of boiled milk with drops of the urine diluted with enough boiled water to provide about three bacteria on the average to each drop, all the glasses soured and curdled within three days.[16]

On the day on which this fact was observed, and when the bacterium was therefore still in full activity, I introduced a small portion of the curd from one of the glasses into a glass of Pasteur's solution, in order to study the behavior of the organism in that fluid. In this experiment I made use of what I have called a "separation tube," a piece of glass tube bent at an angle of about 45° (see Figure 10), with one leg shorter than the other, the shorter leg closely packed with moist cotton wool, *e*, which is purified together with the tube by boiling in water. The apparatus is then transferred to a pure liqueur glass, in which it is arranged with the longer leg, *f*, placed vertically and kept in this position by a mounting of silver wire, *g*, applied to the tube before its purification. The liquid to be experimented on is now poured into the glass to a higher level than the mouth of the shorter leg of the separation tube, but not so high by a considerable interval as the top of the longer leg. The liquid finds its way slowly through the cotton and rises in the longer leg; and it is into the liquid in the longer leg of the separation tube that the

Figure 10

[16]Even after 17 days in the urine the organism still retained the power of causing lactic fermentation, though it was then not quite so energetic, requiring an additional day to produce curdling.

material of inoculation is introduced. The cotton in the shorter leg is so closely rammed that it serves as an effectual filter to prevent the passage of any particles of ordinary solid matter; but if any organism capable of developing in the liquid is introduced into the longer leg, it will work its way through the cotton, and show itself in the general liquid in the glass. Even a motionless bacterium or a filamentous fungus will traverse the closely packed mass, at a rate corresponding with the rapidity of its development. Thus we have a simple means of separating things which are living and capable of developing in any particular liquid from particles which are not alive, or which, though living, are incapable of growing in that medium.

It was into the longer leg of such a separation tube in a glass of Pasteur's solution that I introduced the morsel of curdled milk containing the actively growing *Bacterium lactis*. For a long time it appeared as if no growth whatever of the organism had taken place in its new habitat. But when nearly three weeks had elapsed, thinking that the little bit of curd looked somewhat swollen, I subjected it to microscopic examination, and found that the substance of the curd seemed to have disappeared, its place being occupied by small bacteria having the characters of *Bacterium lactis*, showing that the organism really had undergone some development. But it seemed to have grown only where it had the material of the curd to feed upon; for the outside liquid in the glass showed no trace of any organism either to the naked eye or under the microscope.

Thus it appears that the *Bacterium lactis*, though not destroyed by Pasteur's solution, does not find in that fluid the materials requisite for its nutrition. I also initiated in another glass of Pasteur's solution the experiment of four years before, by inoculating it with urine in which the *Bacterium lactis* was developing actively; but here again, although the specimen was kept 21 days, no sign whatever of bacteric growth appeared. It was therefore plain that the *Bacterium lactis* is really incapable of growing in pure Pasteur's solution.[17]

Thus I was forced to the conclusion that the appearances which I had described in my former communication as due to modifications of the *Bacterium lactis* in urine and Pasteur's solution had been entirely deceptive, and had been occasioned by the accidental accompaniment of other species. And now that we know from the results of experiments like those with the little test tube glasses how very numerous

[17]It is right that I should state that the solution which I employed differed from that made according to Pasteur's formula, in having only half the proportion of sugar, and in having its mineral salts derived from wood ashes instead of from the ashes of yeast. It was, however, with solution of precisely the same composition that I had worked in the experiments of four years previously.

are the bacteria and other organisms which really infest milk, it is easy to understand how such a confusion may have arisen.[18]

Now, however, having good reason to believe that I had got the *Bacterium lactis* pure and unmixed, I proceeded to perform the experiment which constitutes the most important feature of this investigation. I have already described the mode of procedure when speaking of the means adopted for isolating the *Bacterium lactis*. Its object now was to obtain, if possible, absolute proof, which would commend itself to the judgment of all, that the *Bacterium lactis* is really the cause of the lactic fermentation, and no mere accidental concomitant of the change.

On August 30th last—having provided sixteen pure glasses of boiled milk, and having estimated, in the manner already described, the number of bacteria present in every $\frac{1}{50}$ minum of a glass of boiled milk which had been inoculated the day before by touching it with a heated needle dipped in milk curdled under the influence of the pure ferment—I diluted a drop of this milk with boiled water to the requisite degree, and introduced into each of ten of the sixteen uncontaminated glasses a drop calculated to contain on the average a single bacterium, while five of the rest received each a drop supposed to contain two of

[18]It is not, indeed, altogether easy to see what the precise train of events may have been in the experiments of four years ago. I may suggest the following as possible. First, along with the *Bacterium lactis* with which the first urine glass was inoculated, was accidentally introduced another kind of bacterium, which produced the large spirillum-like appearances. This adventitious organism grew freely in the urine, along with the slowly growing and inconspicuous *Bacterium lactis*. It presented considerable varieties of form, some of them closely resembling chains of *Bacterium lactis;* but it was in reality a totally distinct species. Then when the Pasteur's solution was inoculated from the urine, both organisms were again introduced together. Here the spirillum-like organism underwent a modification, assuming the characters of an actively moving double bacterium, a transition which was, I think, sufficiently traced under the microscope See *Quarterly Journal of Microscopical Science* (October, 1873). Meanwhile, the *Bacterium lactis* lay dormant in this medium, but retained its vitality; and by accident it happened that one or more of these still living individuals, lying perhaps at the surface of the liquid in the speck of scum that commonly results from inoculation, was transferred along with the other organism to the last urine glass. Here the latter resumed the spirillum-like form which it had before shown in urine, though retaining now for a while in a limited degree the movement it had acquired in Pasteur's solution. The individual or individuals of the *Bacterium lactis* introduced into the urine along with it were here able to develop again, though slowly, and some of their progeny entered the glass of boiled milk inoculated from this urine glass, and in that medium developed with their accustomed rapidity and with the usual fermentative effect. It may, perhaps, seem trivial to record this complicated hypothetical explanation, but I am induced to do so for the sake of illustrating the excessive care required in order to avoid deception when we are dealing with such extremely minute organisms.

the organisms, and the remaining glass was inoculated with a quantity in which, according to the estimate, there would be four bacteria. The result was that within three and a half days the glass into which four bacteria were supposed to have been introduced contained a curdled mass, and the five which had received the drops arranged for two bacteria each, had all undergone a similar change. Of the ten inoculated with drops averaging one bacterium each, the majority were at this period still fluid; but some assumed the solid condition in the course of the next 24 hours, though at different times. But of this series of ten, exactly five, as it so happened, remained permanently fluid.

This was just the sort of occurrence which might have been anticipated if we believed the bacteria to be really the cause of the fermentative change, and supposed that we had succeeded in forming a fair estimate of their numbers. It was to be expected that the bacteria would not be distributed with perfect uniformity in the water with which the milk was diluted; and hence of the drops containing on the average one bacterium each, some would probably be destitute of the organisms, and the rest have more than one, and in differing numbers, involving slight differences of time in arriving at the stage of the fermentative process which induced coagulation.

But not only were the results of this experiment in harmony with the view that the *Bacterium lactis* was the real fermentative agent; they would, as I believed, afford indisputable evidence of the truth of the theory, provided it should turn out, as former experience made me feel sure would be the case, that every glass which had curdled contained the bacterium, and that every one which remained fluid contained none. Though, as I have said, I did not doubt that this was the state of the case, yet I went through the laborious process of examining the contents of all the sixteen glasses just before leaving Edinburgh, nine days after the time of the inoculation. All those which had coagulated still contained an unaltered white curd with a glistening upper surface, and nothing to indicate the supervention of any change secondary to that of the lactic fermentation. The air in the glass shade of each had the odor of souring; the taste of a little portion of the curd removed for investigation was sour and its reaction sharply acid; and under the microscope, in every instance, bacteria with the characters of *Bacterium lactis* were found, but no other organism. And in the case of the remaining five glasses where the milk was still fluid and unchanged in appearance, the air in the glass shade had merely the slight odor of suet, which Pasteur long since pointed out as resulting from oxidation; the taste was that of fresh milk, and the reaction showed the peculiar form of neutrality which fresh milk exhibits, both blue and red litmus paper acquiring an intermediate purple tint.[19] And on subjecting the contents of each to

[19]Neutral urine presents this same reaction.

will be admitted by all to afford a conclusive demonstration that the particular species of bacterium which we have been studying is really the cause of this special fermentation.[21]

It is true that this proof applies only to one particular kind of fermentative change. But the same method will, I believe, be found applicable in other instances, for the purpose of ascertaining how far the rule is universal that a true fermentation—by which I mean one characterized by the faculty of self-multiplication of the ferment—is caused by the development of an organism. In the meantime, as a contribution of a definite character, so far as it goes, to the elucidation of the nature of fermentative changes, so fraught with interest at the present day alike to the physician and the surgeon, I trust it will be not considered unwelcome to the Pathological Society.

In the final section of this paper, Lister considers the cause of the odor of sour milk (which he believes to be a volatile, neutral substance); the nature of the fermentation process as proposed by Pasteur; and, finally, some of the morphological features of Bacterium lactis. Editor.

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[21]Strictly speaking, the expression should be, not "the cause," but "the cause under ordinary circumstances." For our facts do not of course exclude the possibility of the existence of some other ferment which might produce the same effect upon milk if the circumstances were favorable to its development, as, for example, by the exclusion of the *Bacterium lactis*. In truth, I once obtained, as the result of taking milk directly from the cow into a purified vessel, a minute moving bacterium, which, though it developed very slowly in comparison with the *Bacterium lactis*, produced in time a sour curd, which I have little doubt was due to the change of milk sugar into lactic acid. But thus interpreted, the terms in the text may be considered admissible; just as we speak of the yeast plant as the cause of the alcoholic fermentation, although we know that the *Mucor racemosus*, when growing unmixed in a saccharine solution, gives rise to the same fermentative change.

VIII.

JEAN JACQUES THÉOPHILE SCHLOESING

(1824 to 1919)

Jean Jacques Théophile Schloesing was born in Marseilles, France, on July 9th, 1824. At the age of nineteen he left the Ecole Polytechnique to take a post in the Service des Manufactures Royales des Tabacs, and thus began a long and distinguished career in agricultural chemistry. In 1846 he became director of the Ecole des Tabacs, and shortly thereafter published his first paper on nicotine and its estimation in leaf and manufactured tobacco. In 1866 Schloesing began his work on soil, and from that time until 1879 he opened up no less than five new fields of soil research. His most important work was done on nitrification, and he may, in fact, be considered one of the founders of soil bacteriology. In 1876 he was appointed professor of agricultural chemistry at the newly founded Institut National Agronomique. Eleven years later he followed Jean Baptiste Boussingault as professor of agricultural chemistry at the Conservatoire des Arts et Métiers. Until 1899 he was director of the Ecole d'Application annexée à la Manufacture. Schloesing was elected a member of the Institut de France in 1904. He died in Paris, February 8th, 1919.

The investigation reported here had practical applications in the treatment of sewage, and even more important, proved that nitrification was a microbial process in the soil. The investigation began in 1875 when a commission was appointed to determine the practicality of carrying Paris sewage out to the land between Clichy and the forest of Saint-Germain. Schloesing was asked to draw up the report which resulted in a vastly improved method of sewage treatment.

Although attempts were made to isolate nitrifying bacteria from the soil (in Schloesing's words, "in accordance with the method clearly traced by M. Pasteur for researches of this sort"), thirteen years elapsed before Sergei Winogradsky succeeded in this task in 1890. This must have been a particularly severe blow to Robert Warington, who had begun work on the same problem in 1878. Still, it had been

Institut de France

Jean Jacques Théophile Schloesing. Courtesy R. Courrier.

suggested as early as 1873 by A. Müller that the natural conversion of ammonia into nitric acid was carried out by bacteria.

The following paper by Schloesing entitled "Sur la Nitrification par les Ferments Organisés" was written in collaboration with A. Müntz and appeared in the *Comptes Rendus de l'Académie des Sciences*, Paris, LXXXIV (1877), 301-303. It may be noted that its brevity is inversely proportional to its importance.

ON NITRIFICATION BY ORGANIZED FERMENTS

It is generally acknowledged that the nitrate in the soil arises from the oxidation of ammonia and nitrogenous material of organic origin, but the mechanism of this oxidation is far from clear. Is nitrification the result of a direct reaction, purely chemical, between gaseous oxygen and the nitrogen compounds? Is it the result of the mediation by organisms functioning as ferments? Or is it produced by both of these processes?

These questions have been asked since the day L. Pasteur showed that certain microorganisms, such as the mycoderms of wine and vinegar, have the ability of transferring the oxygen of air to the most diverse organic substances, and therefore are the most active of any living agents for decomposing organic substances. Furthermore, Pasteur proved that organic matter, at least that alterable by those agents in the soil, is singularly resistant to oxygen, when protected by from all living organisms. In reconciling these two facts, one is correct in assuming the intervention of living agents, at least in the case of oxidation and rapid nitrification, without denying that oxygen, active by virtue of chemical and physical forces, has the faculty of oxidizing and nitrifying matter. To investigate these ideas, Pasteur stated in 1862 that the study of nitrification must utilize the new notions on the functions of oxidizing organisms. The results to be reported here confirm the conjecture of the illustrious master and teach the need of rigorous accuracy.

One of us, for purposes of a recent investigation on irrigation with sewer water, needed to know if the presence of the humic matter of soil was indispensible for obtaining purification of this water, that is, total oxidation of the dissolved matter. To this effect, a large glass tube 1 meter long was filled with about 5 kg. of ignited quartz sand, heated to redness, and mixed with 100 gm. of powdered chalk. It was calculated that if this was wetted each day with a constant amount of sewage, the liquid would traverse the tube in eight days. After the

first 20 days, nitrification was not found, and the amount of ammonia in the water was the same; afterwards nitrate appeared, the amount increasing rapidly, and to such a degree that the sewage water leaving the apparatus was devoid of ammonia.

If, in this experiment, the organic matter and ammonia had been oxidized by active oxygen directly, without intermediates, one asks why 20 days were required before oxidation began. If this delay is hypothetically attributed to a process involving organized ferments, then they possibly could not act until fortuitous inoculation and development had taken place.

The experiment, begun in July, had gone on for four months when we had the idea of diffusing chloroform vapors into the tube. One of us had observed that these vapors inhibited all activity of the organized ferments, without impeding the soluble ferments in any manner. If the nitrification observed was produced by organisms, the chloroform would halt it by inhibiting them; if, on the other hand, nitrification was a simple chemical reaction, the chloroform might merely attack or modify it at some point. We therefore placed over the mixture a small vessel containing chloroform, and the vapors were diffused into the tube by a current of forced air. As the daily dose of sewer water required eight days to traverse the tube, and as we did not have time to examine for disappearance of nitrate every day, after ten days we removed the sand and washed it by displacement. The liquid recovered showed no trace of nitrate, but the ammonia in the sewer water was recovered completely. Evaporation of the liquid left a residue of perceptible color and odor, much like that of filtered, but not purified, sewage.

After we had passed chloroform vapors through our tube for 15 days (November 27th to December 12th), we removed the small vessel. For the next 15 days the tube continued to give off the characteristic odor of chloroform. This odor disappeared at the end of December; nevertheless, throughout the course of January, with the tube remaining at 15° C., no trace of nitrate was found. Our nitrifying organisms were all dead, without doubt, and sewer water did not contribute germs for their replacement, possibly because there was a state of advanced putrefaction. On February 1st we tried a new method of inoculating organisms. A garden soil in the process of nitrification might yield them; we therefore added to the sand-chalk mixture 10 gm. of good soil, known by us to possess nitrifying ability, mixed with some water. Nitrate was observed precisely eight days later, on February 9th; the amount has increased since that time, and we believe that the regime prior to the chloroform treatment was reestablished.

It now remains for us to discover and isolate the nitrifying organisms; and we have every hope of being able to achieve this by means of the method clearly traced by Pasteur for researches of this sort.

With respect to the irrigation of sewage, the prime object of our experiment, it was demonstrated that an absolutely sterile soil, sandy and calcareous, supplies a base for nitrification, and serves as an excellent purifier. In an arable soil the oxidative agents and nitrifiers are already present in the soil, and purification of sewage is therefore established the first day of irrigation. In the absolutely sterile soil, purification is retarded until the agents are allowed to develop. It is very probable, simply from the point of view of purification, that this is the principal difference between these two types of soil.

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IX.

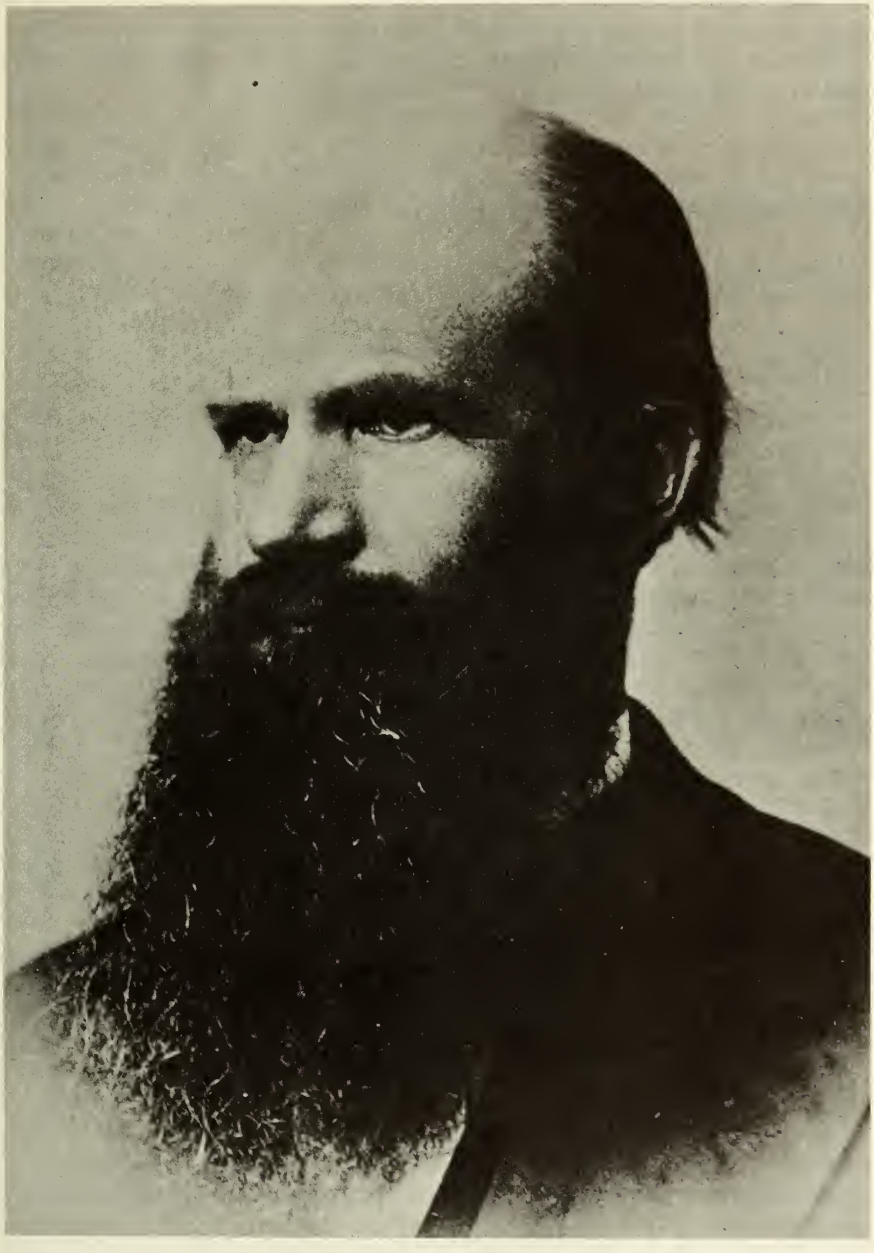
THOMAS JONATHAN BURRILL

(1839 to 1916)

Thomas Jonathan Burrill was born in Pittsfield, Massachusetts, on April 25th, 1839. He received his education at the Illinois State Normal School, from which he graduated in 1865. During the period 1865 to 1870, Burrill was superintendent of schools in Urbana, Illinois. In 1868 he was appointed professor of botany and horticulture at Illinois Industrial University (later the University of Illinois). There he remained until his death.

Burrill's work, largely in the area of phytopathology, began to appear in print after 1877. He was the first to discover and demonstrate a bacterial disease of plants, and in connection with this work he received an honorary Ph.D. from the University of Chicago in 1881. During the years 1902 to 1907 he carried out intensive investigations on bitter rot of apples in southern Illinois. He also investigated ear rot of corn, pear blight, potato scab, rust of blackberries and raspberries, and peach "yellows." Burrill was the first teacher in America to introduce use of the microscope in the classroom laboratory, and was also first to organize a laboratory course in bacteriology. He received an honorary LL.D. from Northwestern University in 1893. He was president of the American Microscopical Society in 1885-86, and at the time of his death, April 14th, 1916, was the seventeenth president of the Society of American Bacteriologists.

In these two papers, published in the *Transactions of the Illinois State Horticultural Society* (XI [1877], 114-116 [New Series], and XIII [1879], 79-80 [New Series]), Burrill introduced a series of studies that were to culminate in his description of the "minute oscillating particles" found in cases of pear blight as *Micrococcus amylovorus* (*Erwinia amylovora*), the etiological agent of the disease. Burrill was successful in transmitting the disease to healthy plants, but he was not too certain of his results; for he wrote: "Does it seem plausible that they (the bacteria) cause the subsequent apparent change? It does to me, but this is the extent of my own faith. We should not say the



National Library of Medicine

Thomas Jonathan Burrill

conclusion is reached and the cause of the difficulty ascertained. So far as I know the idea is a new one that bacteria cause disease in plants—though abundantly proved in the case of animals." This theme is repeated in the chapter on Erwin Frink Smith. The final proof in this instance was supplied by J. C. Arthur in 1885.

REPORT ON BOTANY AND VEGETABLE PHYSIOLOGY

PEAR BLIGHT

It has been quite generally conceded, by those who have kept posted upon the literature of the subject, that this malady is due to the injurious effect of a parasitic fungus. Other theories and speculations are still common, as the effect of frost, or of the excess of heat or moisture, or of a deficiency in the elementary substances of the soil, etc.; but the want of proof in any of these, and the contradictory facts so often presented, prevent the acceptance of any such explanation. On the other hand, those who have carefully investigated, with the microscope, the conditions and progress of the disease, agree in finding evidence of the connection of fungi with the blight of the tree; but no one has yet positively traced what this connection is. I give below, in a few words, the results of examinations made by myself, and extending over several years.

For some time after commencing the examination, no indications were noticed by me which gave any suspicion of the fungus origin of the malady, for reasons now well known, and chiefly because an entirely different kind of parasite was anticipated and searched for from that—or those, it may be—described below. The cambium of the blighted branch, when the trouble first shows itself, and for some days thereafter, is filled with very minute moving particles, very similar to those known as *Spermatia* in fungi and other low plants, and which are now known, through the researches of M. Cornu, of France, to be reproductive, or capable of germinating, and thus giving origin to the plant.

Not unfrequently, a thickish, brownish, sticky matter exudes from affected limbs, sometimes so abundant as to run down the surface or drop from the tree. This proves to be identical with that noticed in the cambium, and unquestionably has the same origin. The sticky, half-fluid substance thus exuding is entirely made up of these minute oscillating particles. The origin of these has not yet been certainly traced; but, if *Spermatia,* as their appearance indicates, they doubtless

are produced in little inclosed conceptacles somewhere in the tissues of the plant.

After some days, the bark of a diseased limb very frequently becomes filled with small papillae, or conical projections, giving the surface a roughened appearance. These originate just under the epidermis, and, by their growth, raise the latter in the manner indicated. Each papilla proves to be a hollow spherical body, with walls of cellular substance, quite distinct from the surrounding tissues of the pear, and bearing upon the inside a coat of hair-like threads pointing towards the center of the cavity, and tipped, when observed at the proper time, with one of the bodies heretofore described in literature as *Spermatia*. There is no question as to these latter organisms being true *Spermatia,* nor is there any doubt as to their production in the bark of the diseased limb; and, when detached from their stalks (the hair-like bodies), they bear every resemblance, in size, motions, etc., to those first described in the cambium. But the latter are confined to the outer bark; the former, except, apparently, as they burst through, to the inner. These appear after the other symptoms of the disease; those before and simultaneous with the discoloring of the leaves. Similar bodies to the last are found on many dead twigs not affected with blight; the first, so far as now known, are not found elsewhere. Are the two in any way connected in origin or growth? Are the later forms products, through germination or otherwise, of their forerunners? We shall know some time, but no answer can be hazarded now.

The above discoveries, if such they are, were made during the warm days of summer; but, in one instance at least, the progress of the blight and the increase of the moving corpuscles were well observed during the warm days of winter, indicating that the disease is not confined necessarily to the time when it is ordinarily observed. In the autumn we have found other forms of fruit very possibly, and almost probably, of the same fungus as that described as showing itself on the outside of the diseased limbs.

One of these later productions would formerly have been classed as a distinct species, under the generic name of *Diplodia,* and consists of papilla-like bodies bursting through the epidermis of the branch, as before described, but bearing inside much larger spores—at first white, translucent, elongated-oval, and of a single cell, but soon becoming thick-walled, dark-brown, opaque, and divided by a transverse septum or partition, appearing much like the final or last-formed spore of the common wheat rust, to which, however, it can have no affinity. These ripened spores, placed in a moist, warm place, germinate in a day or so by the bursting of the outer dark-colored coat, and the protrusion of a white thread common to all fungi, and known as a mycelium. This is at present the end of observations made upon them.

Scattered over the diseased, or rather dead limb, usually in distinct patches from the last, other and smaller papilla-like projections are to be seen very much crowded, so as to nearly occupy the whole surface. Each of these swellings comes from the development of a circle of conceptacles not unlike the last two, but much smaller than the last one. Ten or twelve of these little dark-walled vessels are found in each papilla—each vessel having an elongated neck pointing inward and upward at the center of the papilla, the whole of which is only visible to the eye by close observation. In each of these conceptacles little transparent sacs, filled with very minute curved white pores, are found in great numbers. This belongs to the genus *Valsa,* and no doubt is the perfect fruit of the same fungus to which *Spermatia,* described as being found in conceptacles just beneath the epidermis or skin covering of the bark, and the *Diplodia* conceptacles and spores, also belong. As the apple or pear can be propagated in more than one way, so these minute plants, preying upon the former, are similarly endowed—all three forms noted above being capable, under proper circumstances, of germination, and doubtless propagation of the species; and, if the vitalized specks in the cambium really do belong to the same, still another reproductive form must be recognized.

Still further, during the latter part of summer and throughout the autumn, the leaves and stems of both the pear and the apple are covered by closely felted black threads of a fungus, which apparently lives on the outside of its host, giving the latter the appearance of being dusted with soot. That this is not harmless is shown by the distortion (curling, etc.,) of the leaves, and by their subsequent brittle character, though holding to the last to the tree. This, again, would probably be classed as a separate species; but is probably a form of fungus fruiting ultimately like, in some measure, that last described, and possibly being the same thing. After the leaves fall, this sooty coating is by no means uncommon on the twigs and branches, where a little careful looking will discover it. The threads are very much distorted, bending and doubling and branching in all sorts of shapes, but always with short joints, so as to appear more or less like a string of dark-colored beads, somewhat flattened by contact with each other. It is quite common to find one or more of these jointed threads inside of a hair of the leaf or twig. The thick leaves of lemons, oranges, etc., are very subject to black molds of this kind; and it is well known that with them a form of fruit resembling our *Valsa* is ultimately produced. This favors the opinion of the possible identity of the kinds here mentioned, and also suggests a possible remedy.

If the black, sooty material is the form which, living on the twigs over winter, gives rise to the destructive agent in the hot days of summer, may we not be able to prevent, or at any rate reduce, the loss by disease, by thorough cleansing of these twigs, and the removal

and burning of all dead limbs, during winter time? It will not be an easy matter to wash with alkaline solutions all the small twigs, as well as the larger portions, of fruiting trees; but it can be done, and within the limits of good businesslike culture, provided we can thereby save the trees from this ruthless destroyer. If done before the buds have at all swollen, and so closely wrapped by their protecting scales, very strong material may be used, as strong as the lye used for making soap; and perhaps nothing better than that from wood ashes can be found. The admixture of a little sulfur or carbolic acid could do no hurt; but my observation is, that potash or soda is sufficient, whenever the application can be made directly upon the fungus. The whole matter is exceedingly obscure; but I am well convinced that we shall finally conquer, and that in some way by overcoming the destructive influence of a minute, though sufficiently aggressive fungus enemy.

The twig blight of the apple tree, especially common upon the crabs, is very surely the same thing, or so nearly related as not in some stages to be distinguished; but the white mildew of seedling apples is altogether distinct—this latter being due to *Erysiphe communis*—a not distant relative to that causing the "mildew" of the grapes in European countries. For this, good culture upon dry land, with full exposure to the sun, must be relied upon; but, no doubt, some anti-fungoid material, in powder or solution, may be of service—though just what or how cannot be ventured without practical experiment.

Perhaps it may be worth stating here that none of the diseases mentioned above are due, directly or indirectly, to insects. It is time the talk about the fire blight of the pear being the result of insect depredation should cease. Twigs may indeed be killed by the *Scolytus* species and their allies; but, notwithstanding assertions to the contrary, the appearance of the limbs, with their foliage blackened and dying, and of a peculiarly watery aspect, is, in the case of the real fire blight, so distinct that an accurate judgment may be pronounced while rods away from the tree. Thrips, mites, aphids, etc., injurious enough to the leaves and young shoots, may escape eyesight not trained to look for them; but their work can never be mistaken for the fire blight of the pear, or the too common twig blight of the apple; and the observation must be poor indeed when the work of larger insects cannot be detected upon a short space of a small limb.

No entomologist believes in the insect theory of this blight. Why should it not be given up by other people? In some cases, as very possibly in the scab of the apple, an insect is the first cause, and a fungus the second. But it is not hazardous to state now that insects have nothing whatever to do with the malady here spoken of, as it usually occurs. During the latter part of summer the leaves of apple trees, and especially of seedlings, are sometimes preyed upon by a

small leafhopper (*Cicadellina*) in such numbers that, when disturbed, clouds of them may be seen in the air near the foliage; but these have no intimate connection with the mildew upon the same plants. The case is otherwise with some much smaller two-winged flies; but these prey upon the fungus, not the apple leaves.

The idea that heat, in and of itself, produces blight, is certainly unfounded, since, as noted above, the disease actually progresses in winter, but much more commonly during any temperature of summer time.

A disease of the grape-berry (not *Phylloxera*) has become very injurious in France, where it is known as Anthracnose or Charbon, and is supposed to be the same as our rot. This has been traced to a fungus closely allied to those mentioned above as found upon the dead stems of pears, etc., and helps to justify the suspicion that these fungi, heretofore associated only with dead twigs, may be active agents in some form of causing this condition.

To recapitulate: There is evidence that the theory of the fungus origin of the fire blight of the pear, and the common twig blight of the apple, is well founded; but, though particular species, or what have been regarded as species, are known to accompany the disease, proof has not yet been obtained as to their causing the death of the limbs, nor as to the real action of any fungi upon these limbs. In the meantime, besides every attention to secure vigor and healthfulness of development with little pruning, carefully washing in winter time with a strong alkaline substance in solution is recommended for trial, and as careful removal and burning of every dead limb or twig as soon as observed, winter or summer.

FIRE BLIGHT ON THE PEAR

If we remove the bark of a newly affected limb and place a little of the mucilaginous fluid from the browned tissues under our microscope, the field is seen to be alive with moving atoms known in a general way as bacteria. Sometimes a thick, brownish fluid oozes from the bark of dying limbs and spreads over the outside or falls in drops. This is apparently made up of the living things, myriads of them are to be seen at once. A particle of this viscous fluid introduced upon the point of a knife into the bark of a healthy tree is in many cases followed by blight of the part, but with me not in every instance. The result, if such it be, is not so very uniform as the infection of warm milk; for this, however, there are very possibly other explanations. It is not improbable that certain trees in certain conditions are able to withstand the destroying influences of lowly organized

plant enemies. Plants and parasites are forms of vegetable life; in the struggle for existence one or the other is subdued and the vanquished perishes. If we look once more to the affected branch, we find the disease spreads more or less rapidly from the point of origin; and upon examination the moving, microscopic things are discovered in advance of the discolored portions of tissues, but not very far ahead—an inch perhaps.

Does it not seem plausible that they cause the subsequent apparent change? It does to me, but this is the extent of my faith; we should not say the conclusion is reached and the cause of the difficulty definitely ascertained. So far as I know the idea is an entirely new one—that bacteria cause disease in plants—though abundantly proved in the case of animals . . . . The so-called germ theory of disease in animals, especially in infectious and contagious diseases, is rapidly gaining support and credence. It is not impossible that we are now making a beginning of the application of the theory to the diseases of plants which have heretofore been mysterious and inscrutable.

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X.

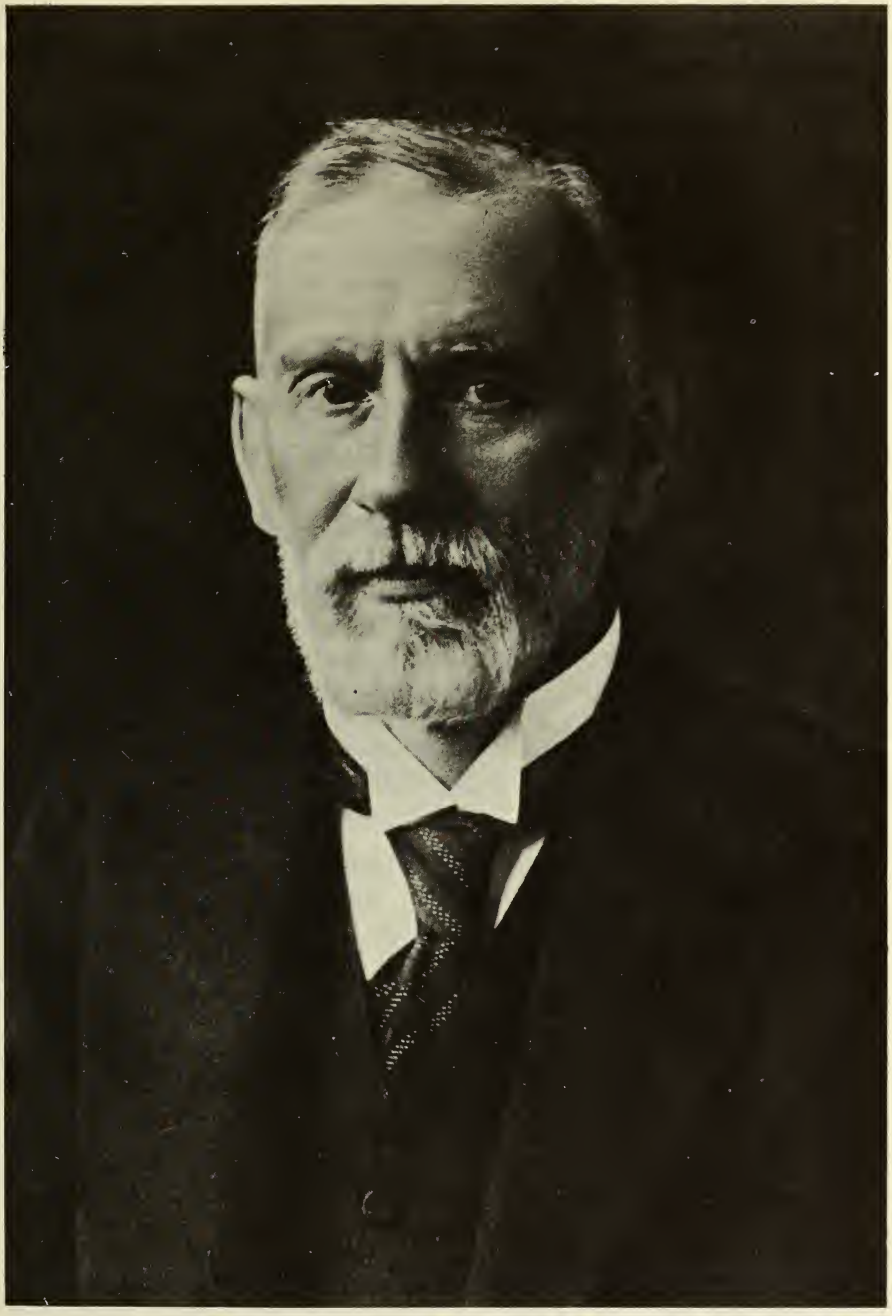
PAUL EHRLICH

(1854 to 1915)

Paul Ehrlich was born in Strehlen, Lower Silesia, Germany, on March 14th, 1854. He received his M.D. degree from the University of Leipzig after previous medical studies at Breslau, Strasbourg, and Freiburg. In 1878 he was made an assistant at the Charité Hospital, Berlin, and he remained there until 1887. In 1890, after spending two years in Egypt to recover from tuberculosis acquired during his experimental investigations, he went to Koch's newly created Institute for Infectious Diseases in Berlin. There Ehrlich developed methods for the treatment of diphtheria. He also was made professor at the University of Berlin in 1890, and six years later he was appointed director of the State Institute for Serum Research and Serum Control. In 1906 he became director of the George Speyer House for Chemotherapeutical Research. He was awarded the Nobel prize in medicine (with E. Metchnikov) in 1908 for his work on immunity. He received honorary degrees from the Universities of Chicago (1904), Göttingen (1905), Oxford (1907), Athens (1911), and Breslau (1912).

Ehrlich was a highly original worker. In addition to his experiments with dyes for differentiating cells and tissues (1877 to 1890), he devised an improved method for staining the tuberculosis organism (*Mycobacterium tuberculosis*). He established hematology as a science by inventing the triacid solution for differentiating the formed elements in blood. He greatly influenced work in immunology (1890 to 1900) with his "side chain" theory, and definitely must be credited with establishing the principles of chemotherapy (1907 to 1915) with his contributions on the treatment of trypanosome and spirochaete infections. Ehrlich is, of course, most famous for his discovery of Salvarsan (606) as a cure for syphilis. Many technical methods invented by him are still in use today.

In 1909 Ehrlich said to his young assistant, H. Bauer, "I should have been a dye chemist." Among Ehrlich's numerous and original discoveries with dyes were the discovery of mast cells, the



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Paul Ehrlich

development of an acid-fast stain for the tuberculosis organism, the methylene blue method for staining gonococci, and a vital staining method. He was, to a great extent, able to establish a fruitful relationship between biology and chemistry. In the following paper, published in the Zeitschrift für Klinische Medizin, II (1881), 710-713, entitled "Über das Methylenblau und Seine Klinisch Bakterioskopische Verwerthung," and written by Ehrlich at the age of twenty-seven, his interest and understanding of the use of dyes as biological reagents are evident. In fact, the next year (1882), he succeeded in staining the tuberculosis organism.

CONCERNING METHYLENE BLUE AND ITS VALUE IN CLINICAL BACTERIOLOGY

At one time, when the question of the pathogenic significance of microorganisms was prominent in medical thought, it was not considered superfluous, since there were but few trained specialists, to present methods to facilitate the detection of such organisms and thus make knowledge of them available to a larger circle. Ignoring scientific aims for the moment, it is sometimes necessary, in the interests of practicality, to be able to determine without doubt the presence or absence of microorganisms in a given case. The following note concerns itself exclusively with this purely clinical objective, and includes only questions that arise in connection with examinations of various liquid exudates and blood.

Each of these studies adds to the evidence that the direct addition of a dyestuff to an organic fluid cannot yield exact results. Dyes are supplied as weakly or strongly alkaline compounds, and the useful ones precipitate in the pure form, so that it is difficult to decide which is dye particle and which is a coccus. We must thank Koch for the technique of carrying out staining procedures accurately, for by his method of drying films to be stained the proper foundation was laid.

Elsewhere I have shown that all available histological pigments, based on their tinctorial and chemical qualities, fall into three groups, and I have classified them as basic, acid, and neutral. For bacteriological studies (on dried preparations) the only suitable group, as far as I see, is that containing the basic dyes. The number of compounds in this group is extraordinarily large, and in each case, either it is necessary to survey the entire group to determine if they are equally suitable or it must be determined whether certain of them seem to be a priori better than the others. In my experience I must choose the latter alternative and state that the usual dyes (Bismarck brown,

fuchsin, methyl, and gentian violet) are not to be recommended. This little note will suffice to explain my view.

As I have stated elsewhere, bacterial staining depends upon the principle of maximum destaining, that is, the bacteria should be stained intensively, and it should be possible to decolorize all other objects readily. In the above-mentioned method of Koch, the condition of maximum decolorization is extraordinarily difficult to attain, and to obtain the correct timing a laborious effort is required.

All of the above-mentioned dyes are very strong and stain even in dilute solutions, and with enough time they diffuse into everything, bacteria, granules, and protoplasm, and eventually even dried serum is intensively colored. It follows that the obtaining of distinct and sharp pictures depends essentially upon the time of exposure to action of the dye. If it acts too long, all elements in the vicinity of the bacteria are stained and thereby masked; if it does not act long enough, the bacteria will stain weakly and be easily overlooked; the optimum point is that at which one neither overstains nor understains. I may add that the concentration of the dye and the nature of the dried preparations influence the optimum staining time, and, further, that some dyes, for example Bismarck brown, precipitate from solution in granular forms; but I believe these difficulties may be eliminated, as will be shown below.

My investigations have concerned the entire series of basic dyes, and I have finally discovered a compound to which the above objections do not apply. It is methylene blue, and it occupies a special position by virtue of its sulfur content. The valuable properties of this dye lie partly in the fact that one obtains a maximum differentiation of microorganisms, especially cocci, and yet it has little tendency to overstain.[1] If one allows a saturated aqueous solution of this dye to act on a dried preparation for a long time, one obtains, even under these unfavorable circumstances, clear and convincing pictures, and both bacterial cells and granules are distinctly and clearly stained. Entirely equal results were obtained with dilute methylene blue solutions if they were given sufficient time (about one hour) to act.

It follows from these observations that in contrast with the previously mentioned dyes, methylene blue is, on the one hand, completely independent of time and concentration and, on the other, allows for maximal decolorization. Wide variations in the composition of methylene blue solutions appear to have only a very small influence on the specific staining mechanism. I found, for example, that saturated

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[1]My experience, as I have stated many times, only partly involves microorganisms affecting man. If I may be permitted to refer to the communication of Koch about six months ago, the dyes I synthesized were used with success in his researches on (animal) infections.

mixtures of methylene blue and equal volumes of glycerin and water gave the same results as simple aqueous solutions. Further comment is not necessary to show that the examination of bacteria using methylene blue, instead of other dyes, is at once easy, simple, and unfailing.

There remains little to observe concerning special techniques. In general, it is best to use a simple, normally dried preparation; one may, particularly if one is working with red blood corpuscles, also use the heating method. After application of the dye—aqueous, moderate concentrations are recommended—and allowing to act for its optimal time (one-half hour to 24 hours), the preparation is washed off with water, blotted gently between blotting paper, air dried, and placed under lacquer and observed with Koch's illumination apparatus and the oil immersion lens.

I have made a number of clinical investigations in the last year and a half using this method, and I should like to mention several of them briefly.

It is my contention, as well as that of others, that negative blood findings cannot be considered of conclusive diagnostic value. Typical of this was a case of ulcerative endocarditis complicated by leukemia, a complete detailed description of which was given under my direction in a dissertation by Dr. Spilling. During the life of the patient only a few cocci could be recognized by careful searching of numerous methylene blue-stained blood preparations; nevertheless, in sections of the spleen of typical homogeneity and density, clumps and masses of cocci intensively stained with methylene blue were found post mortem. This work convinced me that negative results I obtained during an investigation of a case of puerperal sepsis were of only slight significance. In a case of hemorrhagic smallpox I sought in vain for microorganisms in the blood and fresh pustules; this was true as well when I examined cases of scarlatina and the blood and serum of vesicant victims.

The condition of the different cavity discharges is of diagnostic importance. In cases of rheumatic pleurisy and synovitis, as well as fresh gonorrheal arthritis, I have encountered no organisms. In contrast, I have always found micrococci arranged in long chains in the exudates of puerperal sepsis. It is especially important to emphasize that the presence of these organisms does not always lead to suppuration. This is particularly likely in septic inflammation of the joints, because of their serious mucilaginous condition; their pathogenic nature is not revealed without microscopic analysis.

These observations are of prognostic and diagnostic importance, as is shown in this last case.

A woman who aborted (in four months), and required intrauterine attention, became ill with a shaking chill and high fever. In her hospital examination the only condition of importance found was a

hemorrhagic pleurisy on the left side; there was no involvement of the genitalia. At the same time there persisted a high fever (39.5° C.). At the start of my investigations I found absolutely no micrococci or bacterial growth in the exudate, and I accordingly considered the illness as not septic. The course of the illness showed the correctness of my stand.

In closing I should like to state that methylene blue is very well suited for the observation of Neisser's gonorrhea bacterium; and if it is necessary to confirm the specific nature of the gonococcus (Neisser), this dye should be recommended for practical diagnostic purposes.

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XI.

ROBERT KOCH

(1843 to 1910)

The method described in the following paper opened the way for extensive pure culture investigations of the organisms of man's natural environment. Bacteriology owed its phenomenal growth (1880 to 1900) to the many technical methods devised by Koch and his school. In very early work on the "plating" technique the nutrient gelatin was poured (as one would a pancake mix) on cold, sterile, flat glass plates covered with a bell jar (1881). Agar was introduced as a substitute for gelatin in 1882, and the glass plates were replaced by dishes invented by R. J. Petri (1887). The methods used today for studying bacteria in water, milk, and other materials, are essentially unchanged from those devised by Koch nearly eighty years ago. The following paper, originally a lecture to a medical group in 1883, was published in the *Deutsches Ärtzblatt,* CXXXVII (1883), 244-250, and was entitled "Die Neuen Untersuchungsmethoden zum Nachweis der Mikrokosmen in Boden, Luft, and Wasser."

THE NEW METHODS FOR STUDYING THE MICROCOSM OF SOIL, AIR, AND WATER

It is a well-known fact that a number of diseases, especially those that are infectious, are related in obvious ways to air, water, and soil. The concept that has been evolved concerning the manner and mode of this relationship, varies, of course, with each prevailing opinion on the nature of the etiological agent. As long as the currents, temperature, and humidity of the air, the mineral content of water, and the gaseous exhalations of soil were ascribed a dominant

influence on the origin of disease, one was almost exclusively limited to investigating purely chemical and physical qualities of the air, soil, and water.

Later, there was introduced the view that not only does the purely chemical and physical environment cause and accelerate diseases, but also certain natural organic decomposition processes exert much more influence. The result was that the investigator now directed his attention to ascertaining the mode of action of these processes. He endeavored particularly to determine the conditions that inhibited or accelerated them, for example, soil permeability and the kind of ground waters. He sought further for signs of the degree of contamination brought about by decomposition processes, so that a decision as to their injuriousness to health could be made. He sought definite quantities, or at least the levels to be expected, of the carbon dioxide content of air, the amount of oxidizable organic matter in water, and the quantity of ammonia, nitric acid, and chloride therein, and these were taken as fully sufficient evidence of contamination of air or water. The concept of etiological investigations of air, water, and soil was enlarged with important results, for which we have to thank the pioneering efforts of Max von Pettenkoffer, whose contributions are known to all of us.

Recently, further important information has been discovered about decomposition processes. It has been proved that these processes, so similar to fermentations, are governed by simple organisms, and indeed the lowest forms of the plant world, the fungi and bacteria. It will be necessary, in the future, to determine not merely decomposition products but also the causes of these transformations, the microorganisms themselves; and it will be necessary, besides noting the carbon dioxide content of the air, and the quantities of oxidizable organic matter, nitric acid, chloride, etc., to determine the numbers and kinds of fungi and bacteria in soil, air, and water, if we are to obtain an adequate idea of their ill-effects on health.

This concept has lead to many efforts to solve these problems. For such purposes, particulate suspended constituents, spores especially, were investigated by filtering air through gun cotton, then dissolving the latter in ether and examining it microscopically. Air was also driven or filtered against a sticky fluid, such as a drop of glucose or glycerin, on the assumption that the solid constituents of air would remain in the liquid and thus be amenable to microscopic examination. Finally, air led directly into suitable nutrient fluids for bacteria has been studied. It was hoped that all viable spores contained in the air would be caught and then would multiply so that one could determine their relative numbers and kinds. Similarly, water may be examined, either as such or after partial or complete evaporation, by means of methods similar to those used primarily for studying soil particles, for example, with calcium chloride solution, osmic

acid, or substances which stain, such as aniline dyes. I do not know whether similar investigations have been undertaken in connection with soil.

All of these methods are incomplete and uncertain. It would take us too far from our object if I pointed out all the errors in each method; I must restrict myself and speak in generalities. The direct microscopic method for air and water gives no information as to whether the microorganisms are living or dead, and this above all else should be known. The other methods, which bring the particulate bodies of air into a nutrient medium, do not take into account the properties of bacteria relative to their growth and development. Bacteria and fungi do not grow proportionally. Some multiply very rapidly, others slowly. Some are motile, some non-motile. It is possible that any solution, into which ten or twenty different spores from the air are introduced, will within a brief time be overgrown by the motile forms and completely mixed, so that with the confusion of types and forms, one cannot discover how many and what kinds were originally present in the air sample.

The very knowledge of these difficulties is helpful in the search for ways to improve the methods. If one used a solid instead of a liquid medium and deposited a number of spores on it, they would then be separated from one another. The motile forms could not mix with the non-motile, and the fast-growing ones would not overwhelm the slower, because each would be found in a definite place. One could thus eliminate the most important difficulties.

This method is not hypothetical, but rests on experimental proof of which I can give you examples. I must emphasize that once these lower organisms are segregated, further special precautions must be taken for their study.

You know that in air, in water, and on objects, briefly on everything, a number of bacterial spores are present. If one works in an uncontaminated area, one is continuously exposed to the hazard of contamination. One must sterilize all equipment and nutrient substances by heating. All metal and glass instruments and glass vessels are stoppered with cotton and heated to a temperature of 150° to 160°C., using dry heat, and thus are made germ-free. Liquids or other substances that cannot be heated to high temperatures because the changes in their composition would make them unsuitable as nutrients, are heated in flowing steam at 100°C.

I now return to the method of using a solid medium and the germs deposited thereon. Since they are separated, one may observe individual colonies as they develop and thereby obtain information on their properties. An interesting experiment is that in which a boiled potato is cut in half and exposed to the air. The potato is an excellent nutrient medium for bacteria and fungi, but it must, of course, be

initially sterile. This may be done by heating it in a glass tube stoppered with cotton. In this condition the potato could be preserved as long as necessary without change, despite its being an excellent medium for organisms. But if, after several hours' exposure to air, the cotton plug is replaced, there develop a number of germs from the air, and they appear as small points, drops, and fungus mycelium. Almost all forms differ in size, pigmentation, and contour, and if one examines them microscopically, there is no doubt concerning their diversity. Definite information on the germs in the air is quickly obtained by such a simple experiment.

The potato is not suitable for all such work, and in the case of investigations on water or soil it is of no use. Another solid medium is needed, and it is noteworthy that such exists. Gelatin has proved especially useful as a solidifying agent to add to a nutrient solution. One takes gelatin, places it in the nutrient solution, dissolves it by heating, sterilizes it by boiling, and allows it to solidify. One may prepare various types of nutrient gelatin, for example, with an infusion of wheat or dried fruit, the latter being especially good for molds.

A very useful gelatin is prepared with meat infusion, and indeed, no other medium appears so advantageous: for not only do many ordinary bacteria and fungi thrive on it but also, of especial interest to us, pathogenic bacteria grow very well in it.

Peptone may be added to meat infusion gelatin. It is prepared as follows: One kg. of ground beef is mixed with 2 liters of water and allow to stand overnight in a cold room, or better still in an ice chest. Then the water is squeezed out, and gelatin is added in 5 to 10 per cent concentration in 2 liters of liquid (100 to 200 gm. gelatin), 1 per cent peptone (20 gm.), and 0.5 per cent salt. The gelatin is brought into solution by gentle heating. The solution is neutralized and placed in the steam sterilizer. During heating a small precipitate forms which must be filtered, and this is best done with a hot water funnel. After filtration one obtains a perfectly clear, transparent, light yellow solution which soon solidifies. It is possible to introduce germs into the solution during these manipulations, so after filtering, the flask of finished gelatin is stoppered with cotton and heated in the steamer to sterilize it.

The gelatin then remains completely unchanged in the closed flask, in spite of its excellent nutrient qualities, and it may be preserved for a long time. The situation changes if one removes the cotton stopper and allows air to enter. Then, as in the case of the potato, a number of germs from the air fall on the surface of the gelatin and develop. These germs did not arise by abiogenesis from the nutrient substance, or through contamination of the gelatin or apparatus; for in the first case they would be distributed throughout the gelatin, and in the second they would also lie close to the glass walls of the flask.

But they lie only under the opening through which the air entered, and thus they lie on the gelatin surface just as they fell by gravity into the open flask. Germs are deposited when air currents are blown in the flask.

Observation and further study of such colonies is a very different sort of task. Since the colonies in the flask cannot be directly studied under the microscope, it is desirable not to place the gelatin in such a flask, but rather in a vessel with a wide mouth, or on a prepared glass plate. After this gelatin surface was exposed in an open window for two hours, the plate was incubated and the colonies grew out. I noted that some of the single colonies liquefy the gelatin, whereas others do not. Here is a plate on which an entirely different type is represented. This was exposed for one-half hour to the air in the shed of our experimental animals. You see on this a considerable number of colonies with different characteristics from those shown in previous examples.

Many other vessels are more or less suitable for containing the gelatin. Small, square, glass dishes, with a depression and cover, are simple and convenient, and several types are shown here. These were completely filled with gelatin and allowed to stand in the air for several hours. On the surface are various colonies of bacteria and molds. In one of these dishes there is a beautiful brown mold, and in another next to it, almost of the same size, a white one, then a red one, then two yellow bacterial colonies. These have such a graceful and beautiful appearance that one imagines they were made by an artist, rather than accidentally and in a natural manner.

In the investigations of air samples shown up to now, every one failed in determining the exact numbers of germs. In order to do this comparatively, an apparatus was constructed that I will now describe in its simplest terms. It consists of a fairly tall glass cylinder, whose lower part contains, we shall assume, a motionless air layer from which germs may settle undisturbed. Even if air currents pass above the upper opening of the glass, the column of air in the tube will remain undisturbed. Colonies may develop on a gelatin plate placed with forceps in the bottom of the cylinder. If the apparatus is closed up with a cotton stopper, the gelatin plate does not change. But as soon as the stopper is removed, air germs fall on the gelatin and grow, as in these examples taken at various places and various exposure times. I cannot demonstrate all of these to you with the microscope, but I have photographs of enlargements of them, and this will give you an idea of their diverse forms. Their edges appear round, wavy, or else radiate, etc. It is obviously necessary to study these colonies further, using all of the methods and means of new microscopy, with oil immersion systems, illumination apparatus, and various staining methods. One finds that nearly all of these droplets are

pure cultures, and each one may be investigated as a pure culture of a bacterial species. To do this, a platinum needle fused into a glass rod is heated and cooled, then touched to the colony and dipped into a test tube of another nutrient gelatin, which is then stoppered with cotton. There is formed a colony similar in all respects to that of the original colony. A number of pure colonies have been obtained in this manner from the air of Berlin. There are micrococci, bacilli, various aspergilli, and other white, black, and blue-green molds.

This apparatus allows, as stated before, only a comparative estimate of the germ content of the air, and is limited to those capable of developing on gelatin. W. Hesse of Schwarzenburg in Saxony has worked for a long time in the Board of Health laboratory to determine the number of germs in a measured quantity of air.

To solve this problem, it was found that if measured amounts of air are passed over gelatin all the germs are deposited on this gelatin surface. Here is a detailed description of the apparatus used in Hesse's investigation: It consists of a glass tube laid horizontally and coated with nutrient gelatin to one-quarter of its capacity. Both ends of this tube are closed with rubber stoppers. The apparatus is placed on a special stand. A definite amount of air may be sucked through by means of an aspirator. A cotton plug is placed at one end of the tube through which the aspirator passes, and this is wetted with gelatin; therefore, every germ that passes through the tube settles on the gelatin in the first quarter of the tube. The plug at the end serves as a control, for by noting whether there are any bacteria or fungi in it one can tell how completely the organisms have settled on the gelatin in the tube.

Here is a tube through which have passed 10 liters of Berlin air. If you count the colonies, you will find what Hesse established in a large series of experiments, namely, that each 2 liters of air in Berlin contain one mold spore and one bacterium. Through this second tube 12 liters of air were aspirated. This aspiration was somewhat irregular, for there are too many germs at the beginning of the tube. The air, which was very high in germ content, was taken from the animal shed. . . .

These determinations on the ability of air germs to develop have led to several interesting results. As I have already stated, the Berlin air is such that 2 liters contain one mold spore and one bacterium. I have investigated the air of London similarly, and found that approximately fifty-eight more colonies were formed than with Berlin air. The investigation further showed that there was an extrordinary difference in count depending on whether the sampled air was in motion or still. Air can be low in carbon dioxide and still contain a large number of germs. Earlier I found that uninhabited and unventilated rooms are almost germ-free. The air of a cellar may smell musty yet contain only a few germs. Conversely, where there is

much activity and dust, the air contains an unusually large number of germs.

I have already noted the difference between empty and occupied animal pens; in the latter numerous animals were in motion and the air was dusty.

In a study of a schoolroom Hesse examined 2 liters of air before instruction began. It will be seen from his computations that only a few molds and bacterial forms were present. In the same room on the same day 2 liters of air were taken during instruction time. Air taken when the children were leaving the room, and much dust was raised, gave about the same result as that of the occupied animal pens. You will note the great effect of activity on the presence of microorganisms in the air, and on the number of rod forms.

I now come to the investigation of water using these methods. The water bacteria cannot be made to grow on the surface of gelatin as was done with air bacteria. Since gelatin liquefies at 30°C., the water is added at this temperature and carefully mixed. When the mixture solidifies, the germs in the water are separated from each other and individual colonies develop, not on the gelatin surface, but rather within it. Each colony, as shown by microscopic examination, is a pure culture; and as each arises from a single germ, the number indicates approximately the number present in the water. Here you see various dishes of nutrient gelatin to which water of the Spree was added. The gelatin has a large number of small punctiform and spherical colonies. I wish to draw your attention to the gas which proceeds from several of the spherical colonies. The gas is a product of bacterial growth, and it is not unlikely that the development of gases in swamps is a product of definite bacterial species.

Just as in the air investigations, counting and direct microscopic examination, as well as further studies on pure cultures of bacteria from water, are practicable. It is done very simply by adding the water to the fluid gelatin and setting this mixture on a glass plate. For this purpose the plate is placed on a leveling stand in a horizontal position, and the prepared liquid gelatin is poured on. Some germs from the air may fall into the gelatin as it is poured, and this error is difficult to avoid. The number of germs in the air is determined by using one or more control plates prepared in the same manner but with 1 cc. of boiled distilled water added to the gelatin; such a mixture should give rise to from one to five or six colonies. If on a test plate 1,000 or 10,000 colonies develop, the error is quite small. Here are some plates we have made. To this one was added 1 cc. of water taken from the Tegeler water works. You see that the number of colonies is fifty to sixty. This is a rather low number. For comparison, I have this plate, prepared with the same amount. It is easy to count, for the number of colonies is approximately one hundred. Colonies may be counted in one of two ways: If the numbers

are too large to count directly, or if, because of the moisture, a number of colonies coalesce, the colonies on 1 square centimeter are counted, and from this number one can calculate the full number. Here is an example of a bad water. It is a plate containing Spree water. The colonies number in the thousands. However, this water is not so bad as one might think. Here is the worst water, a sample that one will ordinarily not encounter, a drop of Panke (stream) water. This plate shows the colonies developing from a hundredfold dilution. The colonies are nicely separated and easily countable. Additional dilutions could be made to separate individual colonies and thus obtain pure cultures.

Von Rozahegyi of the Board of Health laboratory has made a great number of such experiments. Here are examples of plates he has prepared in his work. This is a plate containing 1 cc. of tap water; these two contain well water. This method for investigating water is very useful in the control of water filtration apparatus. I show here some examples. The first shows a plate with a drop of unfiltered Spree water, and the second a drop of the same after passing through a charcoal filter. After filtration, there is a decrease in the numbers of colonies observed, but it is so insignificant that one cannot think of it as water purification. Only the coarse suspended material with adhering bacteria appears to be held back by the filter, and this is why the number of colonies is decreased. Von Rozahegyi has made counts with filters of the kind used in houses and only in a few cases was the bacterial content in the water markedly diminished after filtration.

In brief, investigations on filters indicate that they may actually contaminate water, because the filters retain particles contaminated with bacteria, and finally putrefaction sets in.

As I have said, pure cultures can be obtained from water as well as from air. Here is a series I have chosen because of their pigmentation. These were collected from Berlin well water.

Incidentally, it should be noted that this counting principle may be applied to various substances, such as milk, or with solid material such as feces.

We now come to the use of the method for the determination of microorganisms in soil. I can speak of this only briefly. One proceeds either as with water, placing the soil in the fluid gelatin, or one spreads it on the surface of solidified gelatin. Here are examples of gelatin plates upon which soil has been spread, and you see the numerous colonies and their characteristic forms. This soil investigation yielded some interesting facts not previously observed. You know C. Nägeli's observation that deep soil layers in the vicinity of ground water are areas of extensive mold growth. I do not know if a positive reason has been given for this. If one investigates soil by our method, the opposite is found, as a few observations will show. Earlier, in other connections, I had investigated Berlin soil, and

indeed this was done as near as possible to the Panke. One might think that soil of this neighborhood, so long associated with sewage, would be infiltrated with putrefaction bacteria. Since I had an opportunity to go there during some house building, I obtained soil samples from various depths. I found, to my great surprise, that only the upper layer, the black earth, in which dung and fallen matter were mixed, was rich in molds and bacteria, bacilli especially. The deeper layers to the water level of the Panke were almost free of bacteria. I also had an opportunity to investigate for one week a drainage ditch in this same vicinity. Here you see tubes of the upper, black layer, samples from a depth of 2 feet, and finally 3 feet. Here are the gelatin plates. The upper earth was rich in bacteria. Nearly every particle of soil has a diverse vegetation of bacteria and mold mycelia. The plate with the soil from a depth of 2 feet does not show much bacterial growth, and from 3 feet the plate contains practically no colonies. Perhaps a few will develop later, but from this investigation it is certain that microorganisms do not occur in large numbers in the deeper soil layers. With the help of this method, we must evolve a new view on the occurrence of microorganisms in soil, which is also a new view on the influence of soil microorganisms on health. The same applies to their occurrence in air and water.

We may now ask, what significance do these findings have? Can it be stated that air, water, and soil contain a certain number of microorganisms and yet are without significance to health? Here I should to say that we have not succeeded in isolating pathogenic bacteria in any of our counts on air, water, and soil, and that even large numbers in these materials cause little illness. On the other hand, we should take into account estimates of ammonia, nitric acid, salt, etc., and then try to clarify the influence of bacterial numbers and quantitative chemical analyses on health, particularly if the numbers of microorganisms are exactly known, even if pathogenic bacteria are not found. It is not unlikely that we may succeed in isolating pathogenic bacteria, for example, the typhoid bacillus in water or soil. Several pathogenic bacteria, among them the typhoid bacillus, the erysipelas micrococcus, and the anthrax bacillus, have been distinguished in pure culture on the same nutrient gelatin used in the air, water, and soil investigations by G. Gaffky. A large series of investigations on the over-all distribution of pathogenic bacteria must be made. The differentiation of simultaneously developing colonies is not difficult. as you can see from these gelatin cultures of typhoid, erysipelas, and anthrax bacteria.

I believe, Gentlemen, that sufficient examples have been laid before you to show that this new method of investigation is of service to hygiene in answering a number of questions on the etiology of infectious disease. It is to be expected that further results will be obtained with this method, and it is important for each doctor interested

in infectious diseases and related questions of hygiene to develop a definite trust in this method of investigation. For these reasons I have, in accepting the honor of this invitation on behalf of the committee, elected to discuss this theme.

Further Reading

Hitchens, A. P., and M. C. Leikind. "The Introduction of Agar-Agar into Bacteriology." *Journal of Bacteriology,* XXXVII (1939), 485-493.

XII.

SERGEI N. WINOGRADSKY

(1856 to 1953)

Sergei N. Winogradsky was born in Kiev, Russia, on September 1st, 1856. He studied for a little over two years at the University of Kiev, first as a law student and then as a candidate in the natural sciences. Dissatisfied with his course and uncertain about his choice of career, he then left Kiev to study music at the Conservatory in St. Petersburg. In 1877, however, he returned to his scientific studies, this time at the University of St. Petersburg. He graduated in 1881, and then, influenced by Pasteur's classical studies on fermentation, he decided to devote himself to the study of microorganisms.

In 1885, Winogradsky accepted an invitation to work under the mycologist Anton De Bary at the University of Strasbourg. Here he carried out his first investigations on autotrophic bacteria and started his work on sulfur and iron bacteria. At Zurich, from 1889 to 1891, he performed major and definitive work on the organisms responsible for the process of nitrification in nature. Although urged by Pasteur to organize a bacteriological laboratory at the new Pasteur Institute, he returned to St. Petersburg in 1891 to work at the Institute of Experimental Medicine, which was also recently established; in 1902 he became its director. Two years later, however, he retired to his estates in the Ukraine and for seventeen years devoted himself to problems of farm management.

Forced to leave Russia after the Revolution of 1917, he decided to resume his scientific career, first accepting a post at the Agricultural Institute of the University of Belgrade. In 1922, however, he received another invitation to join the Pasteur Institute; and from that time until his death, at the age of 96, he lived and worked on a small property of the institute at Brie-Comte-Robert, a few miles out of Paris. During the early part of this period he performed some of his most important studies on the microbiological population of the soil, particularly on nitrogen-fixing bacteria and the bacteria which bring

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Sergei N. Winogradsky

about cellulose decomposition. When the German occupation of France shut him off from the scientific world, he devoted himself to collecting and re-evaluating his lifetime work. In spite of the difficult postwar conditions, this was published in France in 1949. Winogradsky died on February 24th, 1953, the recipient of many honors both from his native country and abroad.

Winogradsky's contributions to microbiology are many and varied. Although he is generally thought of as a soil microbiologist, many of his discoveries cannot be catalogued so narrowly. He did much of the fundamental work on the sulfur-oxidizing, iron, and nitrifying bacteria, and he introduced the concept of chemolithotrophy, or chemotrophy. He discovered the nitrogen-fixing anaerobe, *Clostridium pastorianum (pasteurianum),* in 1893.

In 1887, Winogradsky published a long series of papers on sulfur bacteria in the *Botanische Zeitung,* reporting his discoveries on their true function in nature and their specific physiology. He developed the concept of chemolithotrophy during his studies on the colorless sulfur bacteria. In his work, he used the so-called elective culture method, a tool also used by Martinus Beijerinck, and succeeded in isolating organisms quite special and unusual in their nutritional requirements. The first paper in the series entitled "Über Schwefelbacterien," is presented here. It was published in *Botanische Zeitung,* XLV (1887), 489-507.

CONCERNING SULFUR BACTERIA

The genus *Beggiatoa* includes generally recognized and widespread representatives of organisms which, for reasons to be given later, I propose to designate as sulfur bacteria. These bacteria comprise a very characteristic physiological group in whose metabolism sulfur plays a peculiar role.

Ever since O. Cramer showed in 1870 that the strongly light-refractive granules in *Beggiatoa* filaments were composed of sulfur, these organisms have been repeatedly studied.

Ferdinand Cohn confirmed Cramer's work and found that another entire group of bacteria, *Clathrocystis roseopersinea Monas Okenii, Monas vinosa, Monas Warmingii, Ophidomonas sanguinea,* contained granules completely identical with those of *Beggiatoa.* Cohn investigated the growth conditions of these organisms and attempted to clarify the factors involved in sulfur deposition within the cells, since it is recognized that the phenomenon is unique in the plant kingdom. It surprised him to find that these organisms continued to live in water saturated with hydrogen sulfide, for, he states, "they show an

adaptation to growth conditions that are toxic to all other plants and animals; yet these red organisms (*Monas Okenii, vinosa, Clathrocystis,* and the others mentioned above) appear to thrive exclusively under these circumstances. Indeed one finds *Beggiatoa* and the other sulfur-containing bacteria principally in waters containing dissolved hydrogen sulfide. The *Beggiatoa* are, as long recognized, characteristic inhabitants of sulfur springs; in these they seldom are absent, and they show therein a luxuriant growth not found elsewhere in Nature.

The white gelatinous mass they produce has been known for a long time, and it was assumed to be a lifeless organic substance precipitated from waters containing it *(barégine, glairine).* Regarding this, Cohn was of the opinion that there was a casual relationship between *Beggiatoa* growth and the existence of hydrogen sulfide in water; he conjectured that directly through the metabolic activity of *Beggiatoa* and other sulfur-containing organisms, the reduction of the sulfate in sulfur springs, and generally throughout nature, proceeds with the formation of free sulfur or hydrogen sulfide. Accordingly, these organisms must be able to withstand the toxic effects of hydrogen sulfide, and must possess the ability to multiply normally in oxygen-free water, "for water that contains much dissolved hydrogen sulfide cannot contain free oxygen." In this process of sulfate reduction, sulfur in the form of granules or crystals is deposited within the cells of these organisms. "Finally, it appears that the hydrogen sulfide is absorbed within the cells where it is oxidized."

In regard to the latter proposition, I should like to point out that it is not entirely clear how the oxidation of hydrogen sulfide can proceed concurrently with such an energetic reducing activity as Cohn ascribed to these organisms, because they are supposed to live in water lacking oxygen.

Indeed, Cohn has made no direct attempt to clarify the way in which this reaction is carried out, beyond giving his view regarding the sulfate-reducing ability of these organisms; but he performed an interesting series of experiments which made his concept appear very probable. The first part of the work was done in 1863: He observed that *Beggiotoa* in a flask of aged, hot spring water developed a strong hydrogen sulfide odor. A similar observation was accidentally made somewhat later by Lothar Meyer during an analysis of this same spring water. He found that water with algae *(Beggiatoa)* which had been stored four months in closed flasks developed five times more free hydrogen sulfide than fresh, hot spring water. It seemed to him also "very probable that the hydrogen sulfide of springs is generated by algae."

E. Plauchud has attempted to decide the question experimentally; after a series of studies he obtained the same results as Cohn. A liquid medium, which contained calcium sulfate and a small amount of

organic matter, was inoculated with algae he obtained from a sulfur spring *(sulfuraires)*. Hydrogen sulfide production appeared consistently under these conditions; it ceased if the water was heated to boiling, or upon addition of chloroform, phenol, etc. After heating, hydrogen sulfide production could again be initiated by addition of a minute amount of living *sulfuraires*. This work demonstrated without a doubt that hydrogen sulfide production involved the activities of living organisms, but gave no information regarding the responsible organisms, since no microscopic investigations were made that would prove that under these conditions one of the *sulfuraires, Beggiatoa* for example, would grow well in the experimental flasks, and that the other organisms would not grow at all, or else only in insignificant numbers.

A. Etard and L. Olivier have cultured *Beggiatoa* under different conditions. They observed that *Beggiatoa* filaments, in a liquid medium not containing sulfate, lost their granules but formed them again upon addition of gypsum. These sulfur granules "constitute proof that the reduction is accomplished by the protoplasm of living organisms." E. Duclax, commenting on this work, considered it most probable that the organisms in sulfur springs *(sulfuraires)* reduce sulfate, particularly gypsum, with hydrogen sulfide or sulfur formation. The essential mechanism of the process is still unclear, but during reduction, hydrogen sulfide is produced, which is then oxidized by the oxygen in the air, and then sulfur is deposited in the cells; or is the sulfur formed directly from sulfuric acid? According to Duclaux, the second explanation is likely, for in the first case, that is, in the deposition of sulfur from hydrogen sulfide, we have an oxidation process that does not occur in protoplasm.

In this brief historical survey I have limited myself to the most important work on this question. I may add that Cohn's concept is a dominant one and is cited in several physiological and bacteriological textbooks.

If one attempts to obtain a definite idea concerning the processes on the ground of the above-mentioned experiments, which constitute mere probabilities rather than proof, one sees that we are still in the dark about this interesting question. First, there is no proof that even *Beggiatoa* and other sulfur-containing organisms reduce sulfate, although it is certain that this reduction is brought about directly or indirectly by the activities of living organisms. The luxuriant growth of *Beggiatoa* in liquid media containing hydrogen sulfide may well be the cause of the formation of this gas, as a main part of a reaction sequence; but *a priori* this seems quite improbable, although hydrogen sulfide promotes the development of *Beggiatoa* in some way. The nutritional requirements of this organism are completely unknown and remain open to much speculation. The mechanism of sulfur deposition in the cells is also unclear. If one assumes that the reduced

sulfate is liberated as hydrogen sulfide, then it is difficult to propose that the same is simultaneously oxidized in the cell. Further speculations may be made: Sulfate serves as material for hydrogen sulfide production, on the one hand; for sulfur deposition in the *Beggiatoa,* on the other.

The causes of the two processes do not have to be the same; from a purely chemical point of view *Beggiatoa* may form sulfur by oxidation of hydrogen sulfide, which requires free oxygen, so reducing activity, which proceeds without oxygen, and is involved in the over-all process, is ascribed to other living organisms.

But if all these questions still await an answer, others have not as yet been raised. What significance does sulfur deposition have for the organisms? Are the granules reserve materials or excretions? They must be one or the other. In the first instance, the process of further digestion of sulfur granules is unknown, as is the purpose such a strikingly large amount of sulfur would serve. *Beggiatoa* under the most favorable growth circumstances, as in sulfur springs, are literally stuffed with sulfur granules. If, on the other hand, this mass of sulfur is not used further, it must be regarded as an excretion product, in which case such an overabundance of excretion material in a living cell under normal growth conditions constitutes a highly unusual phenomenon without parallel in physiology.

In the summer of 1886 there appeared E.F.I. Hoppe-Seyler's work on the fermentation of cellulose with the formation of carbon dioxide and methane. This most important and interesting work touched upon the *Beggiatoa* problem. The main part of the investigation concerned the decomposition of cellulose by microorganisms. It was decomposed with the production of equal quantities of carbon dioxide and methane. Hydrogen was not formed. No other constantly produced fermentation products were noted, such as organic acids, humus, etc. Only traces of soluble organic compounds were found in the fermentation liquor. Various bacterial types were found in the medium, the dominant ones being rod-shaped organisms which showed little variation from *Amylobacter,* characterized by P. Van Tieghem as a fission fungus.

The production of carbon dioxide and methane, which Hoppe-Seyler proved to accompany the cellulose fermentation, was brought about by bacteria in water-saturated soil (in the summer), wherever he sought it, thus indicating its wide distribution in nature. An interesting question concerns this fermentation: In the presence of compounds which yield oxygen during the reduction, such as iron oxide, manganese oxide, or sulfate, the proportion of carbon dioxide to methane changed. In the absence of gypsum, iron oxide, etc., it was approximately 1:1, whereas in one experiment in which these were added, the ratio was 10:1. The reason for this difference is that methane arises in the nascent state and reduces sulfate from calcium sulfate according to the equation: $CH_4 + CaSO_4 = CaCO_3 + H_2S + H_2O$. Thus gypsum

was changed with hydrogen sulfide production to calcium carbonate. "Both processes, namely (1) the decomposition of cellulose to methane and carbon dioxide, and (2) the reduction of $CaSO_4$ and Fe_2O_3, suggest independent but related courses, since previously formed methane will not reduce iron oxide or calcium sulfate. This reduction is inconceivable and must occur as a secondary process." One finds similar occurrences in other fermentations which proceed in the absence of air and combined with hydrogen and methane production, as in the fermentation of calcium lactate, protein, etc. Since sulfate reduction cannot occur independently, perhaps *Beggiatoa* is involved. Deposition of sulfur granules within the cell has nothing to do with the reduction process. "The appearance of sulfur granules in the *Beggiatoa* cells proved very definitely that hydrogen sulfide is decomposed during the deposition, and this process can be conceived of only as an oxidation—not as a reduction. The reduction of sulfate takes place in the zoogloic mass of *Beggiatoa,* and they tolerate the hydrogen sulfide thus formed."

Hoppe-Seyler observed that *Beggiatoa* die in the complete absence of oxygen.

I started my investigation in November, 1885, in the Strasbourg Botanical Institute. By the summer of 1886 I had obtained definite results concerning the *Beggiatoa,* namely: (1) *Beggiatoa* play no part in sulfate reduction and hydrogen sulfide formation. (2) Sulfur is deposited within the protoplasm of the *Beggiatoa* in the course of hydrogen sulfide oxidation.

Thus my results are in complete agreement with those of Hoppe-Seyler. Although after Hoppe-Seyler's work the question of the reduction of sulfate and the chemistry of hydrogen sulfide formation must be considered solved, it is not completely definitive, and I shall therefore describe my own investigations in detail, particularly since they are independent of Hoppe-Seyler's and were carried out using different methods.

Beggiatoa cells were the chief experimental material. I shall begin with a report on this organism and then show in what respects it corresponds physiologically with other sulfur bacteria. It would be superfluous to give a detailed description of *Beggiatoa* with drawings, for they have been already excellently described and sketched.

Species relationships within this genus are still uncertain, so nothing will be gained by enumerating and describing the species of *Beggiatoa* that were used. At the end of the paper the reader will find something on the morphology of the organisms used. Here it will be sufficient to state that I worked with completely homogeneous *Beggiatoa* material with a filament diameter of about 3μ and occasionally with a mixture of filaments of various diameters (between 1 and $5\,\mu$).

It is well known that *Beggiatoa* is widely distributed in nature. The genus occurs in almost every swamp and pond, and generally where

there are waters containing decomposing plant residues. *Beggiatoa* cannot always be observed through direct microscopic examination of swamp water or slime, because they occur here only in small numbers, and only by chance have I succeeded in observing a few filaments in the latter. I have found a few such filaments in the ponds of the Strasbourg botanical gardens. In order to obtain sufficient experimental material, I removed water from such a pond, placed slime and plant fragments in it, and allowed it to remain in a room for some time.

Noticeable multiplication of *Beggiatoa* was not observed; in fact, the culture failed. The condition necessary for *Beggiatoa* proved to be a sufficient amount of sulfuric acid salts in the water. Strasbourg water contains only a trace of sulfate. If decomposing plant material is added to spring or swamp water, an odor of hydrogen sulfide develops. I succeeded in obtaining a definite increase in the numbers of *Beggiatoa* by addition of gypsum to the water.

I then proceeded in the following manner: I cut the rhizomes of a water plant (*Butomus* is best) obtained from a swamp into small pieces, and placed them into a deep vessel together with gypsum-containing water. I sterilized these, and covered the vessel with a glass plate. All therefore, was, "ready for the attack." In each experiment I observed the same sequence. After three or four days in a warm room in the dark, the water was hardly turbid; on the surface there appeared *Cladothrix* and green *Oscillatoria,* and always a zoogleic mass of bacteria; after five to six days there was perceptible a weak hydrogen sulfide odor, which soon became quite strong. Careful examination of the depths and the surface of the water revealed no increase of *Beggiatoa,* save for a few filaments here and there. On the surface there were only dead *Cladothrix* filaments and the above-mentioned zoogleic mass; in the bottom of the vessel there were only pieces of *Butomus* and large numbers of various bacteria. Once started, hydrogen sulfide development persisted for a month if the vessel was kept in the dark; otherwise *Oscillatoria* developed along with the bacterial zoogleic mass, and then hydrogen sulfide production gradually ceased. On the surface there was formed a thick white film of sulfur. The formation of hydrogen sulfide and sulfur persisted for a long time, in fact, until the gypsum was depleted. If gypsum was again added, the reaction began anew. On examination of such a vessel from time to time, one observed after four weeks a distinct increase in the numbers of *Beggiatoa.* After approximately two months nearly all of the surface organisms had disappeared, and the *Beggiatoa* had increased extraordinarily; they form delicate white filaments on the walls of the vessel and surface of the water, and clumps can be seen with the naked eye.

This work clearly showed that *Beggiatoa* do not play a part in gypsum decomposition, because their numbers are constant at first, and

increase noticeably only after the process has been in operation for some time. It is very easy to impede the development of *Beggiatoa;* one has only to dip the *Butomus* pieces in boiling water for several minutes and then place them in the gypsum-enriched spring water. An odor of hydrogen sulfide will arise as before, but *Beggiatoa* will not appear, regardless of the duration of the experiment, since they ordinarily do not occur in spring water.

A vigorous development of hydrogen sulfide can be obtained from chopped hay and gypsum water. Hay is finely divided, macerated ten days in water, then placed in a large quantity of water, which is repeatedly renewed, and finally boiled. A handful is then placed in a deep vessel containing gypsum water, and a knife point of swamp slime is added. After two to three days there arises a distinct H_2S odor, and later it becomes very strong. The liquid below the surface remains completely clear, opalescent only in the upper layer during the course of sulfur deposition. On the surface is the bacterial zoogleic mass and a film of sulfur. If one examines the contents of the vessel microscopically, one finds on the surface, as on the bottom, a diverse bacterial flora; neither *Beggiatoa* nor other sulfur bacteria appear during the first 15 days. After this they increase slowly, but after four to six weeks a noticeable increase occurs.

Cohn and Lothar Meyer have observed that when *Beggiatoa* are kept in a closed flask, hydrogen sulfide develops. I have observed that this is a constant occurrence. In May, 1886, I filled five flasks three-quarters full of Langenbrück spring water, and then inoculated them with *Beggiatoa*-containing slime, stoppered them tightly and allowed them to stand at room temperature. I investigated the contents of these flasks microscopically; after four to five days I noticed that the odor of hydrogen sulfide, barely perceptible after two to three days, was now much stronger. Evenutally it became very noticeable, and a thick film of sulfur formed on the surface. After three to four days the *Beggiatoa* appeared as clumps of fixed filaments, many of which were disintegrating. The death of the *Beggiatoa* proceeded rapidly, and after two weeks no trace of them could be found. Hydrogen sulfide generation was apparent for more than one month. The hydrogen sulfide could arise from the reduction of sulfate (of which there was a great deal in the water, during the decomposition of the dead *Beggiatoa*, and partly from the sulfur within the *Beggiatoa*. This is probably the case, for if one cultivates these organisms in water that does not contain sulfate, such as in Strasbourg spring water, one finds no odor of hydrogen sulfide in the presence of decomposing matter, and such is the case also in distilled water. In these instances only the sulfur within the *Beggiatoa* serves as material for hydrogen sulfide generation; the hydrogen that is generated in a nascent state from the decomposition of the filaments reacts with the sulfur granules to produce hydrogen sulfide.

This can easily be demonstrated, as in the following experiment: One takes several (five or six) clumps of *Beggiatoa* filaments, as large as pepper seeds, and places them in a drop of spring water under a large cover glass in such a manner that the filaments are in the center of the preparation. One must proceed cautiously in this operation since many of these unusually sensitive forms die, perhaps even from the pressure of the cover glass; addition of distilled water hastens the death of the filaments. The preparation is placed in a moist chamber. After a time, there appears between the extruded mass of protoplasm from the filaments, an enormous number of bacteria. Simultaneously, around the periphery of the preparation, sulfur deposition begins, and soon the edges of the cover glass are colored yellow-white. Meanwhile, the sulfur granules disappear from the filaments in the center. If one tests the liquid under the cover glass with a strip of blotting paper (paper moistened with a solution of lead oxide in potassium hydroxide and dried), free hydrogen sulfide will be detected. Thus it is quite clear that the sulfur from the dead filaments in the center of the preparation is carried to the periphery by currents in the liquid. This process lasts for weeks, and one may follow it step by step. The strong formation of hydrogen sulfide, which many investigators noted in sealed flasks containing *Beggiatoa,* is thereby clarified. The decomposition of the dead filaments is carried out by living organisms.

I shall now proceed to my studies on the sulfur inclusions of *Beggiatoa,* their nature, mode of formation, etc. In outlining the methods used in this work, I must mention an important detail, commonly overlooked in such investigations. Generally, a study of the nutrients required by lower organisms, their fermentative activities, etc., requires use of the pure culture method, which means that one places the specific organism in a flask protected from chance contamination, allows it to grow, and then determines the chemical alterations in the medium brought about by its metabolic activities. The most important requirement is that the culture be pure, and this is most difficult with *Beggiatoa;* indeed it is unlikely that it can be achieved. *Beggiatoa* cannot be obtained in pure culture by any known bacteriological procedure. The gelatin method, which gives excellent results with some types of bacteria, is not applicable in the case of *Beggiatoa,* for they die soon after the gelatin solidifies. All of my attempts to obtain *Beggiatoa* in pure culture have thus far failed. Although material containing *Beggiatoa* sometimes appears to be pure, it is always contaminated with bacteria; and, as I shall indicate later, the presence of *Beggiatoa* in nature, except in sulfur springs, is governed by the decomposition processes of other bacterial forms. One can never be certain, therefore, that even the smallest inoculum of *Beggiatoa* is not contaminated with other organisms. Thus, in the previously cited work of Plauchud's, in which after intermittent heating of flasks

containing *Beggiatoa,* there always resulted hydrogen sulfide production after a fresh inoculum of filaments, it is evident that bacterial contamination was involved and that the process of decomposition of *Beggiatoa* filaments by these bacteria was again begun.

Doubtless one could succeed in isolating some *Beggiatoa* filaments, but, as we shall see, the conditions for cultivating these organisms are unique, and one would obtain only small numbers in the usual liquid media, and these only after great difficulty. They grow so slowly in comparison with other bacteria that large-scale investigations would be very difficult. I finally succeeded in discovering conditions under which the bacteria usually accompanying *Beggiatoa* grow only very slightly. There is always a possibility that under some conditions these bacteria may actually multiply, and therefore an uninterrupted microscopic control of the culture is absolutely necessary; furthermore, the operation must be carried out in minimal quantities of liquid media.

I am persuaded that only with microscopic cultures can one obtain results worth observing. No special apparatus was required in this work. I placed a clump of *Beggiatoa* filaments in a drop of liquid on a slide, and covered it with a supported cover glass so that a layer of constant depth would be available. Between observations the slide is kept in a moist chamber. The liquid in such a culture may be renewed as frequently as is necessary, for one merely allows a stream of liquid to flow under the cover glass. In this way, bacteria and infusoria are removed, but not the *Beggiatoa* filaments, since they are not free swimming, but rather creep on the glass, from which they cannot be easily detached. They cannot be washed off even with the strongest flow of water. When the necessary conditions are met, the *Beggiatoa* grow excellently. Such a culture may be used for any length of time; I have kept several for two months, during which I had to discard some of the filaments periodically. One can with great convenience and accuracy observe the same growing filament and the effects of external conditions on its development and nutrition; and conversely one may determine the transformations produced in the substrate by microchemical determination.

Let us now return to the sulfur granules of the *Beggiatoa.* These organisms, as found in nature, show a great diversity in their sulfur granule content. The number and distribution of these granules have been considered sufficient to serve as morphological characteristics by several workers; thus Cohn, A. Engler, and G. Winter partly characterized *Beggiatoa* species on this basis. W. Zopf believed that the sulfur content depended on the age of the filament; young filaments were supposed to contain none or only a few sulfur granules. Olivier and Etard stated that with cultures in a sulfur-free liquid medium the granules disappeared, but formed again upon the addition of gypsum. Although they followed this appearance and disappearance, they did not study the conditions under which this occurred.

My work soon showed that the sulfur content of the filaments is not a morphological characteristic; nor does it depend on the age of the filament, but solely on cultural conditions. Depending on these, the filaments could either be stuffed with, or entirely free of, granules. They could go easily from one state to the other. If one takes a small clump of filaments rich in granules, and cultivates them as a slide culture in spring water, the granules are observed to disappear. It is necessary to observe the same filament, and isolated filaments, and to observe the relative number of granules in them. For example: When sulfur-rich filaments were placed in a slide culture in spring water, after 24 hours the same filament contained much less sulfur; and after a further 24 to 48 hours only the slightest trace of granules remained, most filaments being completely empty.* The phenomenon is very striking and is unlikely to be missed in a slide culture.

Later I shall return to this because I wish to show the manner in which the dissolution of the granules occurs, and the significance of the process to *Beggiatoa*. At this point I wish only to mention the fact, because now one is in a position to obtain sulfur-free filaments, and thus study the deposition of sulfur with great accuracy.

The following question was to be answered by direct observation: Does sulfur arise in *Beggiatoa* from SO_4 reduction or from hydrogen sulfide oxidation? To answer this I cultivated filaments both in spring water containing hydrogen sulfide and in the same water containing calcium sulfate. In order to keep the filaments continuously in a hydrogen sulfide medium, I devised the following simple apparatus: the top of a large glass cylinder was closed with a double-holed stopper through which were drawn two glass tubes; one tube extended to the bottom of the cylinder and dipped into a small dish containing water and about one gram of calcium sulfide; the other tube was closed at the upper end with a piece of India rubber tubing, and it served only as an exit for gas when acid was passed through the larger tube. Within the cylinder a strip of moist paper was hung. The cylinder was made air tight by greasing the bottom edge and sealing it with a glass plate. The slide culture was placed at the bottom of the cylinder, and a drop of dilute hydrochloric acid is allowed to flow through the large tube into the dish containing the calcium sulfide; hydrogen sulfide then diffuses throughout the cylinder and into the drop of culture on the slide, and there follows sulfur deposition and oxidation. If one now wishes to obtain sulfur-free filaments, one finds that after three to five hours the filaments already contain numerous granules, which appear as black points under strong magnification; after 24 hours the same filaments are completely filled. If the culture is kept in a moist chamber, the sulfur then disappears from the filaments;

*Winogradsky's illustrations of the experiment are not presented in this chapter. *Editor*.

and to cause it to reappear, the slide must be replaced in the hydrogen sulfide cylinder. This experiment may be repeated with ease if one wishes; two to three hours of weak hydrogen sulfide generation are required, but a vigorous evolution must be avoided, since the filaments are easily killed.

The rapidity and constancy with which *Beggiatoa* deposit sulfur within themselves in a sulfur-containing medium indicate how delicately and uniquely sulfur formation is related to hydrogen sulfide oxidation; for it is unnecessary to assume that the process occurs once through a reduction of SO_4, and at another time as an oxidation of hydrogen sulfide. Nevertheless, I have prepared a series of slide cultures using gypsum-enriched spring water. In such cultures one must avoid bacterial development on dead *Beggiatoa* filaments, for this would result in hydrogen sulfide formation. In successful cultures, the sulfur in the filaments disappears in the presence of gypsum, and as is the case with spring water not containing hydrogen sulfide, sulfur is not redeposited. In the five slide cultures containing gypsum in spring water which I examined for some time, four showed the above behavior. The fifth was different: During the entire observation period (40 days), the filaments remained full of sulfur. I found that this was because a mass of dead *Cladothrix* filaments accidentally were added to the culture, and the decomposition resulted in sulfide formation from the gypsum. I verified the presence of hydrogen sulfide in this culture by testing it with lead oxide paper, but I could also detect the sulfide by odor alone.

Even more convincing results were obtained in a third experimental series in which *Beggiatoa* were cultivated in Langenbrück spring sulfur water.[1] This water contains a sufficient quantity of sulfate

[1]Since I used this water repeatedly during this work, I shall present an analysis carried out on it by Bunsen. This cold, alkaline, salt water, the source of Bad Langenbrück, contained per 1,000 parts:

calcium carbonate	3.4055
magnesium carbonate	2.6503
calcium sulfate (water free)	3.1478
magnesium sulfate	5.0528
sodium sulfate	2.1245
potassium sulfate	0.2072
calcium phosphate (tri-basic, dissolved in carbonic acid)	0.2157
calcium chloride	0.1358
iron sulfide (dissolved in calcium sulfide)	0.0459
calcium sulfide	0.0414
silica	0.0569
free carbonic acid	2.3561
free hydrogen sulfide	0.0994
trace of organic matter and calcium fluoride	
	19.7128

salts, and it was allowed to remain in an open vessel until the odor of hydrogen sulfide had completely disappeared. Then I prepared a series of cultures (eight in all). During the experimental period, half the slides were twice washed with spring water, and the other half were treated identically except that to each 5 ml. of the spring water there was added a drop of hydrogen sulfide-saturated water (such as is used in chemical analysis). In the first series the filaments soon lost their sulfur and formed no more, whereas in the second series the filaments were filled with sulfur throughout the experimental period. The difference between the completely black-appearing, sulfur-choked cells and the colorless, sulfur-free filaments was striking, and this despite the fact that a large amount of sulfate was available in both cases; the difference resulted from the hydrogen sulfide in contact with the organisms in the second series.

Further Readings

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XIII.

SERGEI N. WINOGRADSKY

(1856 to 1953)

Winogradsky began his studies on nitrification in Zurich in 1889. Although many investigators, among them Robert Warington, P. Frankland, and J. H. M. Munro, had attempted to isolate specific nitrifying bacteria, their methods failed because they were based on procedures used mainly for isolating pathogenic bacteria. Since Winogradsky had already studied two groups of bacteria which required inorganic compounds as energy sources, it soon became apparent to him that he was working with a third group possessing a similar physiology. It is interesting to reflect that only a little more is known about the nitrifying bacteria today than when Winogradsky made his contributions.

The detailed paper presented here is Winogradsky's first report on nitrification published in the *Annales de l'Institut Pasteur,* IV (1890), 213-231, and entitled "Recherches sur les Organismes de la Nitrification." He had already published two brief notes in the *Comptes Rendus de l'Acadêmie des Sciences* (Paris) in the same year. With this paper and three others, also written in 1890, he laid the foundation for subsequent thinking on problems of plant nutrition, sewage disposal, marine life, and the transformation of inorganic nitrogen compounds by bacteria.

INVESTIGATIONS ON NITRIFYING ORGANISMS

The introductory seven pages of this paper contain a critical review of the evidence for nitrification by microorganisms, or a "nitric ferment," presented in papers by J. J. T. Schloesing and A. Müntz, W. Heraeus, B. Frank, L. Adametz, P. and G. Frankland and others.

Winogradsky shows that whereas in some of this work the presence of a nitric ferment is suggested, all the experiments have technical or logical flaws. Editor.

One sees from this historical survey, now concluded, that positive results were not obtained in any recent work on the subject of nitrification. In addition, the cooperation of living agents in the phenomena has been questioned anew, but since the work of Schloesing and Müntz, there is no doubt that living agents are involved.

Is it necessary to deny the existence of a nitric ferment because of experimental failures, as did Frank? Quite the contrary, these failures render the existence of such a ferment more probable, but the results indicate that nitrifying ability is rare in the microbial world, and most common soil organisms are not endowed with this property. Consequently, though the active agent is doubtless living, one is forced to conclude that nitrification is a function of only a few microbes, which to the present time, have escaped investigators.

I was convinced of this after my studies of the two groups of organisms which I termed sulfur bacteria and iron bacteria, the physiological properties of which are known to the readers of the *Annales*. These are the studies that led to the question with which we are concerned at this moment. If there are organisms which have an exclusive role of oxidation to sulfuric acid, and others which have adapted their functions to the oxidation of ferrous salts, one is obliged to believe in the existence of more specialized organisms exploiting as an energy source, by oxidation, the ammonia of soil and natural waters. My previous experiments with respect to the significance and importance of the phenomenon of oxidation for living beings suggested to me that the same essential relationships were incontrovertibly present in the nitric ferment.

One may attribute the series of negative results to the imperfection of the methods used for isolating the desired microbes. This idea will probably appear audacious to the modern bacteriologist, who relies on possessing certain methods, believed infallable by some persons; but one method, excellent for some one purpose, is of no use for another. Some bacteria do not develop on a gelatin medium, and they are not few in kind. I have had this experience with the oxidizing organisms mentioned about, and it seems plausible that they bear a similarity to the nitric ferment. Probably few people are aware that errors may originate from this fact.

One begins, for example, a bacteriological analysis of water or soil, using formulations given in treatises on bacteriological methods, by mixing the material with nutrient gelatin poured in plates. When it is a case of enumerating organisms present in the mixture, the numbers obtained may be somewhat smaller than there are actually, but in the present case, where the research is done on a specific microbe of unknown nature, one risks a great deal in using this

procedure. It is completely contrary to the pursuits of bacteriologists to find that the nitric ferment does not form colonies on gelatin. We shall understand this when we further analyze the work on this organism. A bit of soil introduced into an ammoniacal liquid may bring about an energetic nitrification, but when this is transferred to gelatin, only the ineffective organisms grow. The most plausible explanation is that one has involuntarily eliminated the nitric ferment, which does not thrive on gelatin. This is most likely the explanation of Heraeus' experiments, and one wonders why this idea did not occur to him.

These considerations induced me to lay aside at the beginning of my research all formulas, and to proceed slowly, but certainly, from there. The discovery and isolation of a nitrifying organism is not a simple task, and some of my efforts, however unsuccessful, are of interest from the point of view of methodology. It is for this reason that I shall describe all the steps in my work, without, however, entering into details in all cases.

I started with the following plan of attack:

1) To obtain the most favorable liquid nutrient, with cultural conditions favorable for nitrification and unfavorable for reducing phenomena.

2) In holding these conditions constant, to make a series of sufficiently long-term cultures to eliminate all species not adapted to the conditions favorable to nitrification.

3) Having ascertained that the growth of the cultures is pure, and that there are no further changes, the nitrification remaining very active, to proceed to the isolation of *all* species found and determine their nitrifying power in pure culture.

The first nutritive liquid employed was a mineral salts solution: potassium phosphate, magnesium sulfate, potassium carbonate, ammonium chloride for nitrification, and potassium tartrate as a carbon source. To start the course of nitrification, I used two samples of Zurich soil; one rich in organic matter, the other poor in organic matter but rich in carbonates. I introduced different quantities of these into plates of large diameter, in which there was a shallow layer of nutritive liquid.

The first experiment was not successful. There was no nitrification of the liquid. After six days there was not even a weak reaction with diphenylamine. I was suspicious of this delay. Apparently the cultural conditions were unfavorable for the action of the nitrifying organisms. I varied the degree of alkalinity, the amount of ammonium salt, and I used different ammonium salts, all without success. It then occurred to me to remove all organic material, and there resulted an immediate intense nitrification.

Thus, finally, it was discovered that a medium rich in organic

matter is not satisfactory for nitrification. Heraeus had observed as much in the fact that its addition, at least to mixed cultures, was not favorable for nitrification and I know of at least one organism which grows well in liquids almost devoid of organic matter. It is evident that this is the case with nitrifying organisms, and one cannot doubt that organic matter impedes nitrification.[1]

Thereafter, I used only solutions of inorganic salts and very pure water (from Lake Zurich). There is reason to believe that nitrifying organisms are supplied with all the necessary substances, since their nitrifying action did not become weak in the course of a long series of experiments.

I shall repeat that the use of earth carbonates, particularly magnesium carbonate, was most propitious for the phenomenon, and I adopted the simple fluid given here:

Ammonium sulfate	1 gm.
Potassium phosphate	1 gm.
Lake water	1,000 cc.

Each large plate contained 100 cc. of this liquid and, in addition, 0.5 to 1 gm. of basic magnesium carbonate suspended in a little distilled water, and this formed a milky solution which was sterilized by boiling.

In this medium, nitrification regularly occurred. Following inocu- of a small drop of nitrified liquid, it was observed that after four days there was a good reaction with diphenylamine; in two more days the reaction was so strong that one drop of the culture liquid transformed a number of cubic centimeters of diphenylamine to an inky blue-black color. In 15 days no ammonia was detected. There were some exceptions (which I will discuss later), but generally the regularity of the phenomenon was gratifying, and I was able to consider the first part of my task accomplished. The same liquid not inoculated, and subjected to the same conditions, did not give a diphenylamine reaction after two weeks.

I now began an examination of the organisms that grew in these cultures. On direct microscopic examination, I saw that there were several species present, but when the liquid was plated on gelatin, numerous other organisms were revealed. Some of these grew rapidly on the solid medium and liquefied it. These were of little interest, and they tended to disappear upon successive cultures in the liquid medium. Meanwhile, I was able to identify others as known species that did not nitrify in pure culture.

After nearly three months, the number of species in the liquid appeared constant. Careful microscopic study of these species in a

[1] I shall not dwell on an exact interpretation of this fact, which may have various explanations.

drop of nitrifying liquid revealed the colonies in the same relative numbers. Evidentally all were adapted to the prevailing conditions, very poor in organic matter, very rich in ammonia; and if they did not have nitrifying powers, at least the numbers one had to deal with were reduced by this mode of culturing.

At this point I attempted to culture all of these species and to test their action in the pure state on ammonium salts. One naturally uses such methods without absolutely relying on them. The possibility that the nitric ferment would prove refractory to cultivation was present, and I submitted the liquids to direct microscopic examination frequently, and by such procedures clarified the problem.

I shall not discuss the species isolated by the gelatin method. They are not of much interest, for their nitrifying powers were absolutely nil. I shall content myself with enumerating them with letters of the Greek alphabet:

- α, a micrococcus;
- β, an interesting small *Oidium,* forming rose colonies on gelatin;
- γ, rods, long and thin;
- δ, rods, short and thick;
- ϵ, a strange organism, apparently not a bacterium but a budding form (Sprosspilze).

Microscopic examination of liquids undergoing nitrification presented nothing really characteristic. The liquid covering the layer of magnesium carbonate was usually perfectly limpid. On the surface appeared an extremely fine film which was difficult to see. When nitrification was most active, generally from the sixteenth or seventeenth day, one could detect a slight turbidity in the liquid. This turbidity, or rather feeble opalescence, was transient. It was due, as I saw with the microscope, to oval organisms, and a few fusiforms that moved with great agility in the liquid. The coincidence of the appearance of this motile phase and active nitrification was noted many times, and I could not but conclude that it was apparently the nitric ferment; nothing more could be said regarding its disappearance from the liquid, or the consequences of it.

It was obvious that the organisms oxidizing ammonia should be sought out in the surface film of the liquid; the behavior of the mycoderm of vinegar and other organisms suggested this idea. Studies of the organisms on the film were conducted with great care, but the ones found principally were *Oidium* β, with which others of the species enumerated above were mixed. But β and the others all gave negative results, and there were definitely no other organisms in the film.

I at once modified the experiment and used a liquid rich in ammonia, containing from 0.5 to 10.0 per cent ammonium sulfate; or I added a standard solution of this salt to a liquid undergoing nitrification. I hoped in thus prolonging the process of nitrification to induce

the multiplication of the causative organism and thereby facilitate its discovery.

I shortly found the key to the problem.

My attention was drawn to the layer of basic magnesium carbonate in the bottom of the plate. I perceived that this salt, at first finely divided and perfectly white, alters markedly in older cultures. The color changes to ash gray, and its consistency becomes gelatinous. If one allows this layer to remain at rest for one or two weeks, no turbidity results, and the gelatinous membrane prevents the carbonate from clouding the liquid. If this membrane is torn, the material in the layer is found to consist of a mass of ash-gray flakes. Microscopic study of these reveals an interesting structure. They are composed of transparent lumps of salt, literally covered with a thick mass of oval bacterial cells. They resemble perfectly in form and size the organisms in the previously mentioned motile phase, and thus its significance was not misunderstood. If one adds dilute acetic acid, the salt disappears, the flakes lose their whiteness and become ash gray and transparent, but keep their exact shape; a quite characteristic zoogleic mass marks the places of salt dissolution. One obtains the impression that these formations are not accidental mixtures of microbes and salt, but are due to the action of the organisms which fix themselves on the particles and engulf them in the gelatinous matter they secrete. There is but a trace of organisms on the surface of the liquid, none on the walls of the container; most are concentrated in the carbonate base layer.

There is heavy growth in this part of the medium during the process of nitrification; by comparison the liquid is practically devoid of growth. Thus it was very probable that I had the nitric ferment. I found a fact to support this belief.

I have already mentioned irregularities in experiments on nitrification. Sometimes the phenomenon began late or not at all, although nitrification was expected after the surface of a nitrifying liquid (limpid at this time) had been touched with a drop of inoculum on a platinum wire. I changed the method of inoculation by using a freshly drawn and sealed capillary tube, placing it in the liquid, breaking its point against the walls of the vessel, withdrawing some small flakes, and then depositing them on the base of a fresh medium. Nitrification began after 24 hours, and after three or four days I again obtained a blue-black reaction with diphenylamine. After this change, there were not many failures and the phenomenon was induced with perfect confidence.

It was still necessary to isolate the organism in question and prove by an irrefutable experiment that it was the nitric ferment. But how to proceed? To this point it had not been grown on a solid medium. I tried again, and added a large number of flakes. These were taken from the base material in the above-mentioned way, and agitated in distilled water, then removed and added to a flask of liquefied

gelatin. Such flasks contained millions of cells. After the fourth day organisms in the flask were recognized as those of the surface film; the hyaline colonies of γ, the granular ones of α, the clusters of β, etc., grew very slowly. The latter remained for a long time. I tried other solid media of different composition, but they were all as unfavorable as the nutrient gelatin.

I decided, therefore, to use the solid medium, not as a direct means of purification of an impure inoculum, but rather to eliminate the ϵ organism from the liquid medium. The idea occurred to me to use a liquid medium without a trace of organic matter. Initially, it contained very little, and the ϵ organism really did not thrive. Still it remained living, and multiplied with each inoculation; then multiplication stopped, evidentally with the complete exhaustion of available organic matter. On the other hand, the nitric ferment grew as before.

I pass now to my experiences with mediums made with distilled water. I prepared a solution containing an ammonium salt and free from all traces of organic matter, a task not so simple as one might believe. This is not the place to discuss the details, so I shall say only that the ammonium salts commercially available though reputedly pure, contain organic bases, and I prepared my own absolutely pure ammonium sulfate. With carbonate bases, one uses calcined calcium carbonate freshly saturated with carbonic acid.

The results of this experiment were surprising. *Nitrification was not delayed, and it proceeded with the same intensity as previously.* Microscopic examination revealed good growth of the oval microbe in the carbonate base layer. All other organisms had disappeared. After the second inoculation, I saw (by inoculation to gelatin plates) that there was a complete absence of the cells designated as α, β, γ, δ. But species ϵ held on. In attempting to exhaust this by a series of cultures in the same liquid, I failed, and finally gave up attempts to free the nitric ferment from ϵ by this method.

If one takes a drop of this last culture, after shaking, and mixes it with nutrient gelatin, one does not obtain colonies for six days. At the seventh, colonies are visible, but they grow very slowly. It was ϵ, in an absolutely pure state. The organism involved in nitrification was not revealed. Thus I was assured, by repeated experiments, that ϵ was not really involved in nitrification. Its multiplication was completely without significance, and the numbers did not exceed 20 per cc.

I proceeded in the following manner to isolate the oval microbe in base layer. It has been amply demonstrated that the procedure of culturing on gelatin is of no use in revealing its presence. Very well, there are better methods for uncovering the nitric ferment from a mixture of microbes if it is present. I no longer had doubts concerning the reasons for the failure of my predecessors.

It was at least very probable that I had something besides ϵ to

which the appreciable nitrification activity could be attributed. The ferment was maintained in this sufficiently pure state for nitrification experiments in liquids poor in organic matter.

It was necessary to separate the nitric ferment from ϵ. After all the purification work, the idea occurred to me to use the negative quality of plating for this task, since the nitric ferment did not grow on nutrient gelatin. One employs the method in the reverse sense, that is, to reveal contamination in the ferment rather than the ferment itself.

The following procedure was used: Some material from the base layer was removed with a capillary tube—this contained lumps of calcium carbonate and the zooglea of the ferment—and it was added to sterile distilled water; this material was again taken up with a pipette, and a certain number of drops allowed to fall on the surface of a plate of spread solid gelatin.[2] The gelatin absorbs the water of the drops, but the site is marked by the calcium carbonate crystals. One allows these plates to stand for six days at 18°C., and then subjects them to microscopic examination. (There are always many drops that do not show colonies.) At once one removes some of this calcium carbonate residue to new inoculating liquid.

In this way I inoculated six base layers, and in all I found nitrification, although some took as long as three weeks. The cause of the delay is without doubt due to the smallness inoculum, and the ill state of the nitric ferment after this treatment. After one month all plates showed measurable amounts of nitric acid. With one exception all were free from ϵ. To prove this, I again removed some flakes from the base material and, after shaking thoroughly, inoculated them on gelatin. This test remained completely sterile. I had therefore finally isolated the nitrifying microbe.

Here is a summary of the results of work reported in this paper:

1) Schloesing and Müntz had attributed nitrification to a special microbe, a nitric ferment, which resided naturally in soil.

2) This microbe was isolated, and it developed profusely in an appropriate liquid, wherein it exercised its proper function.

3) The contrary results of my predecessors are easily explained by their use of experimental procedures inappropriate to the task.

In speaking of a *nitric ferment,* I must conclude by stating that I do not believe there is only a single species able to exercise this function on the entire world's surface. This appears improbable to me. I believe it is rather better to characterize it as a *physiological type.* As to morphological properties, it is probable that these vary, so that it will be necessary to distinguish species and varieties. But there

[2]In this experiment I used small crystallizing disks with covers, employed for the first time, I believe, by R. J. Petri.

will probably be only a few. In Zurich, for example, I have found only the one just discussed. It is remarkable in that it resembles forms found in the soil in extreme eastern Europe. The studies pursued here may clarify some of these questions.

The purpose of this first report was to answer the question: What is the active cause of nitrification in the soil? I have stressed that the method I followed here is indispensible in microbiological work, and of this I assure the reader. I shall not further discuss the nitric ferment here. There should be no doubt as to the intensity of the nitrifying action of my microbe. Is it really responsible for this phenomenon in the soil? I believe that it is. In comparing the amount of ammonia nitrified per day in my cultures with that in the experiments of Schloesing with soil, I say that he obtained, not more, but rather less on the average. I shall present evidence of this in my next report.

XIV.

ROBERT WARINGTON

(1838 to 1907)

Robert Warington, a chemist's son, was born in Spitalfields, just outside London, on August 22d, 1838, and died at Harpenden, Hertfordshire, on March 20th, 1907. As a boy, he studied chemistry in his father's laboratory and, in fact, received most of his early education from his parents. In 1859 he began work as an assistant to Sir John Lawes at the Rothamsted Experimental Station, but illness forced him to leave in January, 1860. In 1862 he went to the Royal Agricultural College, Circencester, first as an assistant to Dr. A. Voelcker, and later as a teaching assistant to Professor A. H. Church; and it was here that he published his first paper dealing with the quantitative determination of phosphoric acid. Warington left the college in June, 1867, after an unfortunate disagreement with J. Gilbert concerning publication of some of the latter's experimental data. During the period 1862 to 1867, Warington published four papers on the influence of ferric oxide and alumina in decomposing soluble phosphates and other salts and their retention in the soil. In 1863 he was elected to the Chemical Society.

After leaving the Royal Agricultural College, Warington became a chemist in the tartaric and citric acid factory of Lawes at Milwall. Here he stayed until 1876, when Lawes suggested that he work with him as his assistant at Rothamsted. After a short tour of the German agricultural experiment stations, Warington returned to Rothamsted and began a brilliant career which marked him as one of the "first generation" of great agricultural workers of this institution.

Up to this time, Warington's chief contributions were in perfecting analytical methods for use in agricultural research. In 1877 he turned his attention to the subject of nitrification, a problem on which he worked until 1891. His principal publications on this work appeared in the *Journal of the Chemical Society,* and bear the title, "On Nitrification," Parts I to IV.

It has been suggested by A. Müller in 1873 that the conversion of ammonia to nitrate was carried out by microorganisms, and in 1877

The Royal Society of London

Robert Warington. Courtesy of I. Kaye.

Schloesing and Müntz (see Chapter VIII) proved this experimentally. Warington verified the work of the French investigators in 1878, and in 1879 showed that nitrifying organisms required calcium, potassium, and phosphorous. He obtained liquid cultures which converted ammonia to nitrite (1879), and cultures which converted nitrite to nitrate, but which were unable to oxidize ammonia (1881). In efforts to isolate the responsible organisms, Warington studied bacteriological techniques at the Brown Institution in London (1886). Although he never succeeded in this project, Warington's results were valuable. He showed that many, but not all, organisms from the soil were active denitrifiers, and more important, that one species of organisms alone could not be responsible for the process of nitrification, that is, the same species could not oxidize both ammonia and nitrite. During these investigations, Warington was elected to the Royal Society of London in 1886.

Warington's failure to isolate the organisms he had obtained in enrichment cultures as early as 1879 was doubtless due to his ignorance of their physiology. His lack of experience in bacteriology led him to try techniques used for the isolation of pathogenic bacteria. As Winogradsky brilliantly demonstrated, organisms concerned with nitrification do not thrive in highly organic media. Furthermore, Winogradsky had previous experience with organisms of similar characteristics (chemoautotrophic bacteria). During the work on the nitrifying organisms, Warington made extensive determinations on the nitrate content of Rothamsted soils. He presented these results in a lecture to the Society of Arts, for which he was honored with a silver citation. Warington retired from Rothamsted in 1891.

After his retirement, Warington was named under the Lawes trust as American lecturer, and he presented six lectures on nitrification before the members of the United States Department of Agriculture. In 1894 he was made Sibthorpian Professor of Rural Economy at Oxford. He retired to Harpenden in 1897 and remained there until his death.

The following paper is a summary of Warington's work on nitrification as it appeared in the *Journal of the Chemical Society,* LIX (1891), 485-529. It is numbered Part IV, the other three parts appeared in the same journal: XXXIII (1878), 44-51; XXXV (1879), 426-456; and XLV (1883), 637-672.

ON NITRIFICATION

As I am obliged for the present to discontinue my researches on nitrification, it is the object of the present paper to gather together the principal unpublished results which I have obtained. Many lines of investigation will be found incomplete, and some results stand in need

of verification, but their publication may be of some value to other investigators. The facts I have to mention will be more conveniently grouped under distinct heads than narrated in chronological sequence. The first section deals with experiments made long ago, with more or less imperfect methods.

The first part of the original paper (pp. 485-494) deals with the chemical reactions and the distinction involved in the production of nitrites and nitrates in soil; and with the establishment of the concept of a "purely nitrous agent," and the factors affecting it. Editor.

The Isolation of the Nitrous Organism

Original Description of the Nitrifying Organism

J. J. T. Schloesing and A. Müntz, to whom we owe the proof that the process of nitrification is determined by a living organism, claim to have separated that organism from all others, and have given a description of its appearance under the microscope.

The method used for its isolation consisted simply in the preparation of successive cultures in sterilized liquids suitable for nitrification. With the experience since obtained, it seems unlikely, though not impossible, that a pure culture would be obtained by this method: moreover, no proof of purity is given, save that the final culture contained but one kind of organism when viewed under the microscope. Their description, however, of the appearance of the organism agrees well with the characters since ascertained in cultures of greater claim to purity, and there can be little doubt that they actually observed the true nitrifying agent. What they have told us left, however, so many questions unanswered, that it was essential, for an exact knowledge of the subject, that the organism would be carefully isolated and further studied. The isolation of the organism was not only required by the bacteriologist; it was also most important that the chemist should be able to study its properties when separated from the multitude of other organisms with which it is associated in soil.

According to the original description of Schloesing and Müntz, the nitrifying organism consists of corpuscles, round or slightly elongated, of varying dimensions, largest when grown in mediums rich in organic matter, but at all times very small, occurring either singly or in pairs, multiplying by division. A. Müntz and V. Marcano have since stated that the nitrifying earths of Venezuela contain in abundance an organism three or four times larger than that just described, but after cultivation in weak sewage, the dimensions approximate to those of the French organism. The tropical organism apparently possessed peculiarly energetic nitrifying powers.

E. Duclaux has made a careful study of the nitrifying organism as separated by the French chemists. He speaks of it as having a feeble

refractive power; as not being truly round or oval, but more like a bacillus broken short, with all its angles rounded; and, further, as exhibiting a difference of size in the same culture greater than that shown by any other organism he is acquainted with. He naturally suggests that more than one species may be present.

Earlier Rothamsted Work

My own work was originally undertaken solely from a chemical point of view, and previously to 1887 I had not the command of a microscope suitable for bacteriological work; some facts relating to the development of the organism in nitrifying solutions were, however, observed, and a few microscopic observations were made by friends to whom materials were submitted.

It was soon noticed that when nitrification had energetically set in, the small quantity of precipitated calcium carbonate placed at the bottom of the bottle assumed a curdled appearance, hanging together in flakes. When the supply of calcium carbonate was insufficient, it was entirely dissolved as nitrification proceeded, and small flocks of gelatinous matter remained at the bottom of the culture. The same gelatinous matter could be produced by treating the curdled sediment with dilute hydrochloric acid. Both the curdled sediment and the gelatinous matter were microscopically examined by Mr. G. H. Makins, of St. Thomas's Hospital, a former pupil of Dr. E. Klein. Under date April 20th, 1883, I find in my notebook: "Solution and sediment examined. Masses of highly refractive corpuscles, circular, sometimes two in contact, often in a mass of zooglea. These bodies not altered by HCl, not stained by iodine, stained feebly by alkaline methyl-blue."

In 1884, nitrification was started in several pretty strong solutions of ammonium carbonate, no solid calcium carbonate being present. These alkaline solutions remained quite transparent during nitrification. A good while after the conclusion of the experiment, clear, gelatinous flocks were observed at the bottom of the solution. Circumstances here seemed peculiarly favourable to the occurrence of the nitrifying organism in tolerable purity. The gelatinous matter was stained and examined by Mr. Makins; it consisted of a mass of small, generally oblong corpuscles, very rough looking and ill-defined. No other forms were seen.

In 1886 I went for a short time to study under Dr. Klein, at the Brown Institution. The culture containing the gelatinous matter just named was taken with me. The substance was spread on gelatin; a small bacillus was obtained, which I have called *Bacillus tardicrescens* [*Xanthomonas tardicrescens*]. This bacillus was introduced into suitable ammoniacal solutions, and found to possess no nitrifying power.

In the light of recent work, it is easy to see that the gelatinous matter I examined was in fact the nitrifying organism. S. N. Winogradsky,

in his first memoir on nitrification, has described the formation of these gelatinous clots, and from them he finally obtained the organism in a pure condition. The ill success which attended my attempts at isolation was due to the now well-recognized fact that the nitrifying organism will not grow on gelatin.

The next stage of my investigation has been already described to the Society; it consisted in the examination of a number of organisms, including several reputed to possess nitrifying powers, with the view of ascertaining whether they were capable of nitrifying ammonia. The investigation was from this point of view entirely unsuccessful. Dr. P. F. Frankland, and several other investigators, have met with the same result.

Recent Rothamsted Work

In March, 1889, I commenced a systematic attempt to isolate the nitrifying organism; I was wholly unaware that Dr. P. F. Frankland was occupied with the same subject. The point first aimed at was to prepare a series of cultures of the nitrifying organism under conditions unfavorable to the development of ordinary bacteria, and thus either to effect their final exclusion or at least so to reduce their proportion that the final separation of the nitrifying organism should be made much easier. The condition I chiefly relied on for effecting this separation of organisms was the exclusion of organic matter from the solutions. J. H. M. Munro had shown the possibility of conducting nitrification in solutions to which no organic matter had been added; experiments of my own, to be described furhter on, had confirmed this fact. It will be convenient if I mention at starting the nature of the media employed.

MEDIA EMPLOYED

As microscopic examinations of the solutions would be necessary, I determined to discard my old culture fluid containing ammonium chloride and solid calcium carbonate, and to substitute a solution of ammonium carbonate; a clear solution without sediment was thus obtained. The composition of the solution was as follows:

Water	1,000.00 ml.
Ammonium carbonate	0.25 gm.
Ammonium chloride	0.50 gm.
Potassium phosphate	0.04 gm.
Magnesium sulfate	0.02 gm.
Calcium sulfate	0.02 gm.

The ammonium chloride was merely added to prevent the precipitation of magnesium and calcium phosphate. The solution was placed in wide-mouthed, stoppered bottles; these were adopted to prevent loss

of the ammonium carbonate. The bottles were half-filled. After sterilizing and seeding, they were covered with paper caps, and placed in an incubator kept at about 22°C.

Besides other ordinary liquid media, a special one was prepared containing the soluble organic matter of soil. An arable soil was exhausted of nitrates by the application of cold water on a vacuum filter; the soil was then boiled with water and filtered; the clear, amber-colored solution obtained then received ammonium chloride, phosphates, etc.

A considerable variety of solid media was employed in the course of the investigation. The ordinary 10 per cent gelatin, made with beef broth and peptone (Gelatin P); a 10 per cent urine solution, solidified with 6 per cent of gelatin (Gelatin U); a solution of 1 gm. of asparagine, 0.5 gm. sodium acetate, 0.5 gm. potassium phosphate, 0.2 gm. magnesium sulfate, 0.2 gm. calcium sulfate per liter, solidified with 6 per cent gelatin (Gelatin As). The last two with 1 per cent of agar-agar substituted for the gelatin (Agar U, Agar As). A solution of 0.4 gm. ammonium carbonate, 0.2 gm. potassium phosphate, 0.1 gm. magnesium sulfate, 0.1 gm. calcium sulfate, solidified with 6 per cent gelatin, or 1 per cent agar-agar (Gelatin AC, Agar AC). All these media were made faintly alkaline. Later on, the ordinary nitrifying solution, containing ammonium chloride and solid calcium carbonate, was solidified with 6 per cent gelatin (Gelatin ACH).

The object of employing these different media was to supply, if possible, the conditions most favorable to the growth of the nitrifying organism.

FIRST SERIES OF EXPERIMENTS

While waiting for the main series of cultures to be sufficiently advanced for examination, some work was done with a culture in the ordinary ammonium chloride solution, seeded from a culture two years old. The culture examined was a third successive culture in solutions free from organic matter; the product of nitrification was nitrite. The sediment in this culture was examined by streak and plate cultivation on Gelatin U, Gelatin As, and Agar U. The result was the isolation of two bacilli, which I have called *Bacillus patulus,* and *Bacillus oleosus.* These, and all the other separated organisms obtained in this investigation, I hope to describe in a future paper.

The trials of the nitrifying power of these two bacilli will be mentioned further on.

SECOND SERIES OF EXPERIMENTS

The first culture in this, the principal series of experiments, was started on March 28th, 1889, with a little unmanured arable soil. A normal nitrification occurred, nitrates being finally produced.

Second cultures, seeded from the first, were started on May 9th and June 27th. The solution used in this and subsequent cultures was the ammonium carbonate solution already described. The product of the nitrification in these cultures was only nitrite; the power of producing nitrates was thus already lost.

A third culture was started on August 16th. A fourth on September 19th. A fifth on October 3d. A sixth on October 25th. None of these cultures exhibited any turbidity.

Several of these cultures were subjected to microscopical examination. The lens used was a Leitz $\frac{1}{20}$ immersion, giving a magnification of 800 diameters. In some cases, a few sterilized cover glasses were placed at the bottom of the bottle in which nitrification took place. At the end of nitrification, the liquid was removed with a pipette, the bottle and cover glasses were dried at 35°C., and the cover glasses taken out and stained. This plan was proposed by Mr. G. H. Makins; it was suggested by the fact that the nitrifying organism always collects in large numbers on the bottom of the vessel in which it grows. It is interesting to note that the organisms in the bottle lost all nitrifying power by drying at 35°C.; when the bottle was refilled with a fresh ammoniacal solution, no nitrification took place.

The sunk cover glasses were found covered with masses of corpuscles, usually oval, and having a length generally exceeding 1μ; the other forms present were in very small proportion. Stained preparations made from the nitrified solutions showed comparatively few organisms. The forms met with in the whole of this series of examinations were: (1) The corpuscles already mentioned. The larger ones were frequently rough in outline, reminding one of the rough, rounded masses of a siliceous sea sand; these did not stain deeply. There were usually a few smaller oval corpuscles, regular in form, and always longer than broad. (2) Some very small circular organisms, often appearing as mere points, staining much more darkly than the preceding. (3) A few slender bacilli staining faintly. (4) Occasionally forms like *c,e,s.** I did not feel certain that these were a distinct organism, but thought they might be mere broken shells of the large corpuscles.

The third, fifth, and sixth cultures were examined as to the growths they would yield on gelatin and agar. Following Klein's method, a loop of platinum wire was dipped into the nitrified solution and then rubbed over a sloping surface of gelatin in a test tube; by this method the organisms grew as distinct colonies, as in a plate cultivation. Plate cultivations were also made in the ordinary way. As no actively liquefying organism was present, it was possible to continue the observation of these cultures for many weeks. All the cultures gave an abundant growth on gelatin. The two bacilli separated during the first series of experiments were not found in these purer cultures. A small bacillus,

*Figures not presented in this chapter. *Editor.*

staining somewhat feebly, was practically the only organism obtained. I have called it *Bacillus frugi*. A liquefying coccus and a torula were obtained in the course of plate cultivations; but as only single colonies of each occurred, they were probably of atmospheric origin; nevertheless, their power of causing nitrification was thoroughly tested.

The results of the gelatin and agar cultures appeared remarkable; although the composition of these media had been specially arranged, so as to agree as far as possible with the conditions suitable for the growth of the nitrifying organism, no growths were obtained on gelatin or agar-agar corresponding to the oval corpuscles which formed by far the most abundant organism in the nitrified solutions. Since, however, several of the solutions yielded apparently no organisms on gelatin save the small bacillus, and yielded this abundantly, it seemed possible, as was suggested at the time by Dr. Klein, that we had to do with a polymorphic organism, and that the bacillus obtained was really the nitrifying agent.

Each of the five organisms which had been separated from nitrified solutions, including the two which were more probably atmospheric contaminations, were sown into solutions suitable for nitrification: (1) The usual ammonium chloride solution. (2) A similar solution containing soil extract. (3) An ammonium carbonate solution. (4) A 1 per cent urine solution. These solutions were seeded both with the solid organisms obtained from the surface of gelatin and with broth cultures of these organisms. In some experiments mixtures of several, sometimes of all five, organisms were introduced, it being thought possible that nitrification was a complex reaction effected by the joint action of several agents. Besides the separated organisms, the growths on gelatin obtained immediately from the nitrified solutions were employed. In no single case in the whole series of trials did any nitrification take place.

As growth on the surface of gelatin took place under conditions very different from those which occurred when the organism was submerged in an inorganic solution, it was thought possible that the power of nitrification might be lost by cultivation on gelatin, and could be only gradually regained. The gelatin cultures were, therefore, sown in broth; the broth cultures were used as seed for an ammonium carbonate solution containing 20 per cent of broth; these cultures employed to seed similar solutions containing 5 per cent of broth; these to seed solutions with 1 per cent of broth; these to seed the usual inorganic solution of ammonium carbonate. In no case was nitrification obtained.

Attempts were also made to obtain nitrification from the gelatin cultures by seeding them onto a moist, well aerated, solid medium, the object being to imitate the conditions occurring in a natural soil. Stoppered bottles were half-filled with small pieces of marble free from dust; the marble was moistened with an ammonium carbonate solution containing 1 per cent of broth; the solution did not cover the

broken marble. When sterilized, these bottles were seeded from the 5 per cent broth cultures mentioned in the preceding paragraph. In no instance was nitrification obtained.

In a few cases the gelatin cultures prepared from the nitrified solutions were grown out of contact with air, the solution being applied to the lower part of a sloping surface of gelatin, and then the upper portion of the gelatin carefully melted and allowed to run over the seeded surface. Growths were obtained, but they failed to yield nitrification.

The fifth and sixth of the successive nitrifying cultures in inorganic solutions were sown on November 19th, 1889, in weak broth, at a temperature of 20°C. The broth became turbid in six days. When 18 and 20 days old, stained preparations were made, both from the liquid and from the white deposit at the bottom of the test tubes. Some of the stained preparations seemed to consist entirely of *B. frugi;* in others a longer bacillus was also present. None of the oval corpuscles were seen, but the deeply stained, minute dots mentioned above were certainly present.

The fifth and sixth cultures were also sown in a solution of ammonium carbonate containing 20 per cent of broth. These solutions actively nitrified. From these, solutions containing 5 per cent broth were seeded. From these, solutions containing 1 per cent broth were seeded. From these, cultures in solutions of ammonium carbonate were prepared. The whole of these solutions nitrified readily; the solutions were always turbid. Stained preparations showed that the bacilli diminished as the proportion of broth decreased, while the deeply stained minute dots became more manifest. None of the oval corpuscles were seen.

TRIALS WITH THE DILUTION METHOD

While the gelatin cultures obtained from the nitrified solutions were being examined, an attempt was made to separate the nitrifying organism from the others associated with it by means of the well-known dilution method. One part of the third successive culture in the ammonium carbonate solution was mixed with 500 parts of boiled water, and one drop from a capillary tube was added as seed to each of five bottles containing the ammonium carbonate solution. This was done on September 28th, 1889. After 41 days, solution No. 5 was found to have nitrified, the others showed no change. After 91 days, Nos. 2 and 3 were found nitrified. Two solutions did not nitrify; it therefore appeared as if the dilution used had been sufficient.

The three solutions which nitrified all gave growths on gelatin. Growths took place more speedily on Gelatin U and As than on Gelatin P. The organisms obtained on gelatin were sown in appropriate liquid media, but no nitrification was obtained.

On January 14th, 1890, ten tubes, containing an ammonium carbonate

solution with 5 per cent broth, were each seeded with one drop of a dilution to 1:1,000 of a cultivation in ammonium carbonate and 20 per cent broth, seeded from the sixth successive culture of the original ammonium carbonate series. Of these ten solutions, none nitrified.

On March 13th, Professor P. F. Frankland and Mrs. Frankland communicated to the Royal Society a paper on "The Nitrifying Process and Its Specific Ferment." They had started, like myself, by preparing a successive series of cultures (twenty four) in an inorganic ammoniacal solution. From these cultures they had obtained growths on gelatin, none of which possessed any nitrifying power. They had finally, after many trials, succeeded, by using a dilution of 1:1,000,000, in obtaining a nitrified culture which gave no growth on gelatin, and which they believed contained the nitrifying organism in a pure state. In a note published in the *Chemical News* of March 21st, I briefly stated my own attempts to isolate the nitrifying organism up to that date.

On April 16th, 1890, I made a fresh attempt with the dilution method. A subculture from solution No. 2 of the first dilution series was diluted to 1:1,000, 1:10,000, 1:100,000, and 1:1,000,000; each of these dilutions was seeded into five stoppered bottles, containing an ammonium carbonate solution. The bottles were placed in an incubator, and maintained at about 22°C. The solutions were tested up to 190 days, but not one of them nitrified.

On considering the possible cause of failure in the last two attempts I had made with the dilution method, it occurred to me that it might be due to the alkalinity of the ammonium carbonate solution employed. It was quite true that this solution was easily nitrified when seeded in the ordinary way, but as alkalinity, when beyond a trifling amount, is a condition which hinders nitrification, it seemed possible that it might prevent it altogether when the nitrifying agent was reduced to a very few organisms. In former experiments on the distribution of the nitrifying organism in the subsoil, I had found that the addition of gypsum to the diluted urine employed enabled me to detect the presence of the nitrifying organism by its action on the solution at far greater depths than when the urine solution contained no gypsum, and therefore became alkaline before nitrification. This fact had not been forgotten in preparing the solutions for the last series of trials by the dilution method; the strength of the ammonium carbonate solution had been reduced to one-half for the two stronger dilutions, and to one-quarter for the two weaker. I now resolved to abandon the ammonium carbonate, and to return to my old ammonium chloride solution, containing solid calcium carbonate. The solution employed by Frankland was of a similar nature. My solution contained in one liter:

Ammonium chloride	0.20 gm.
Potassium phosphate	0.06 gm.
Magnesium sulfate	0.03 gm.
Calcium sulfate	0.03 gm.

This solution was placed in twenty stoppered bottles, which were half-filled. The bottles were divided into four series, A, B, C, D, each of five bottles, which were respectively seeded with one drop from a capillary tube of dilutions to 1:1,000, 1:10,000, 1:100,000, and 1:1,000,000 of a second subculture of No. 3 in the first dilutions series. The experiment commenced on June 3d, 1890.

Tested after 35 days, no nitrification had occurred. After 66 days, nitrification was in active progress in A1, A4, A6, and had just commenced in B1, B2, B5. After 91 days, nitrification had commenced in B3. After 115 days, nitrification was found in progress in C3, C5, and D4. Nitrification thus occurred in ten solutions. No other solutions nitrified.

Each of the nitrified solutions was spread on gelatin, both Gelatin P and Gelatin U being employed. It was found, as before, that growth took place far more easily on Gelatin U than on Gelatin P; indeed several solutions gave no growth on the 10 per cent gelatin-peptone, which yet after eight or nine days produced a considerable growth on the 6 per cent gelatin-urine. On ascertaining this fact, the whole of the remaining testings were conducted with Gelatin U.

Out of the ten nitrified solutions, there were three, B3, C3, and D4, which gave no growth on Gelatin U, either when spread upon the surface, or when introduced into the substance of the jelly. Thus, early in October, 1890, I became possessed of three solutions which probably contained the nitrifying organism in a pure condition. I had no time to ascertain by a further extension of the dilution method whether these cultures contained more than one organism; I at once proceeded to experiment with them.

After the publication of Frankland's paper, and before my own successful result, Winogradsky commenced the publication of his "Recherches sur les Organismes de la Nitrification." In his first memoir, he describes the method employed for isolating the nitrifying organism. After a series of cultures in an inorganic ammoniacal solution, by which the organisms growing upon gelatin were reduced to a single species, he collected the gelatinous clots which appeared at the bottom of the culture vessel, washed them, and spread them upon gelatin. After ten days, he observed where no growth had taken place, and from these places proceeded to seed fresh ammoniacal solutions. He succeeded in this way in obtaining actively nitrifying cultures which gave no growth on gelatin. In a fourth memoir, recently published, he describes a better plan of isolation by growth on the surface of gelatinous silica. A solution of dialyzed silica is concentrated till the addition of a saline solution determines its gelatinization. The solution of silica is then poured into dishes, and rapidly mixed with one or two volumes of a solution containing ammonium sulfate, potassium phosphate, magnesium sulfate, calcium chloride, and sodium carbonate (or magnesium carbonate. The mixture becomes solid in 15 minutes. On

this medium apparently all the organisms which flourish in inorganic solutions can be grown, but no others. The nitrifying organism gives, according to Winogradsky, very characteristic colonies upon silica.

Properties of the Nitrous Organism

The organism isolated by the dilution method converts ammonia into nitrous acid only; it is, in fact, the agent with which we have become familiar in the earlier part of this paper. Frankland had arrived at the same result. It was, indeed, to be expected, as only nitrites were produced in the cultures from which it was separated.

With the pure organism, a number of solutions of potassium nitrite, containing phosphates, etc., were seeded. Some of these solutions were supplied with monosodium carbonate and carbonic acid, others with calcium acetate, the object being to supply the conditions most favorable for the oxidation of the nitrite. In no case was any diminution of the nitrite perceived.

Some experiments were also made to test the reducing power of the organism. Müntz apparently thought it possible, from facts which he observed, that the nitrifying organism when not in contact with air might act as a reducing agent. Two test tubes, about two-thirds filled with broth containing calcium nitrate, were seeded with the nitrous organism on March 26th, and kept at 22° C. The solutions were tested on May 9th, but no nitrite was present. Stained preparations proved that the nitrous organism was present, though not abundantly. I may here mention that none of the bacilli growing with the nitrous organism in the inorganic ammoniacal cultures has any power of reducing nitrates to nitrites.

I am unable to verify Frankland's statement as to the behavior of the nitrous organism in broth. He says, "On inoculating from the pure nitrifying solutions into broth, the latter develops after a considerable time (about 20 days at the ordinary temperature), a very characteristic growth, the liquid becomes turbid, a very thin, whitish pellicle forms on the surface, and afterwards a considerable amount of glutinous deposit collects on the bottom, the whole liquid in fact becomes highly viscous, and adheres to a needle in long strings." I have in twenty-four instances seeded test tubes of broth with the pure nitrous organism. The broth was made with 1 lb. of beef to a liter of water. It was employed both neutral, and of various degrees of alkalinity. The cultures were kept at 22° C. In no case did the broth become turbid, nor did any visible growth appear. As already mentioned, the organism could be found in the broth, but it apparently had not greatly multiplied.

It has been already shown, by the experiments made during the isolation of the nitrous organism, that it refuses to grow on gelatin. When the ammoniacal solution, in which the organism freely grows, was solidified with 6 per cent of gelatin, no growth was obtained by seeding this with the pure nitrous organism.

It was of great interest to ascertain whether the nitrous organism was itself capable of attacking nitrogenous organic bodies, or whether it was necessary that other organisms should first reduce these to ammonia before nitrification could take place. On obtaining the organism in a pure state, experiments were at once started in solutions containing urea, urine, asparagine, and milk. The urine was a 1 per cent solution. The milk contained 5 cc. of sweet skim milk per liter. The urea solution contained; urea, 0.50 gm.; potassium phosphate, 0.10 gm.; magnesium sulfate, 0.10 gm., per liter. The asparagine solution contained; asparagine, 0.2 gm.; potassium phosphate, 0.10 gm.; magnesium sulfate, 0.10 gm. per liter. All the solutions received precipitated calcium carbonate before sterilization. Two experiments, each seeded from a different culture of the nitrous organism, were made with each solution at the temperature of 22°C. Afterwards a more extensive series of experiments was started with similar solutions at the same temperature, six bottles of each solution being taken, each six containing three pairs, each pair seeded from the same source, but one bottle receiving calcium acetate, 0.50 gm. per liter. The results of the two series may be summarized together.

Out of the eight asparagine solutions, seven showed a very distinct nitrification. One of the nitrified solutions, which had remained quite clear, was spread on gelatin; no growth was obtained. The calcium acetate appeared neither to facilitate nor to retard the nitrification.

Of the eight milk solutions, five showed distinct nitrification. One of these was clearly an impure culture; it became very turbid, a thick film formed on the surface, and it gave an abundant growth on gelatin. Three of the nitrifying cultures, which had remained without any increase of the natural turbidity of the milk, gave no growth on gelatin. The calcium acetate was apparently without influence.

Of the eight urine solutions, six showed decided nitrification. One of these was contaminated with mold, but gave no growth of bacteria on gelatin. Two of the clear nitrifying solutions were spread on gelatin, and gave no growth. The calcium acetate exerted no beneficial influence.

Of the eight urea solutions, only three have nitrified; one of these was tested on gelatin, and gave no growth.

These experiments should be regarded as preliminary only, as the course of nitrification has not been studied, or any quantitative experiments made. That the pure nitrous organism can attack nitrogenous organic bodies is, I think, fully proved. The nitrification observed has however been, especially in the case of asparagine and milk solutions, decidedly sluggish. The contaminated mlik culture has nitrified to a much greater extent than the corresponding pure culture. It is quite possible, therefore, that other organisms may render considerable service in the process of nitrification, by making a preliminary attack upon organic matter.

Form of the Nitrous Organism

The description of the nitrifying organism given by the French observers has been already noted.

Frankland's description of the nitrous organism when grown in an ammonium chloride solution is as follows: "The bacilli are about 0.8μ in length, and hardly longer than broad; in fact their form is intermediate between that of a bacillus and micrococcus, so that the term bacillococcus may not inappropriately be used to designate them. They occur both isolated, in pairs, and in small irregular groups." Grown in broth, they form "small bacilli, about 1.5μ in length, and about 0.5μ in breadth, sometimes, forming threads 5.7μ in length in which the divisions are not generally apparent."

According to Winogradsky, the organism growing in an ammoniacal solution has generally the form of an elongated ellipsoid, but the youngest cells are nearly spherical. The breadth does not exceed 1.0μ, the length is 1.1 to 1.8μ. The longest are those about to divide, and exhibit a dumbbell form. More rarely the organism is spindle-shaped with blunt ends. A chain of three to four individuals is very rare. When in repose, the organism is united by a jelly, and loses all characteristic form. The organism has a temporary mobile stage of existence, during which the culture may become turbid.

Before mentioning the forms which have come under my own notice, I will say a word as to the mode of staining. The simplest, and one of the most satisfactory methods, when merely eye observations are desired, is to place a drop of the culture liquid on the glass slide, mix with it some filtered stain by means of a wire, place a cover glass on the drop, and allow it to stand about half an hour; then press down the cover glass, wipe off the liquid which exudes, and finally run Hollis's glue round the cover glass. By this means, the organism is stained in its own culture fluid; we see it in its true form, without any possible distortion of its shape by drying. The field is bright and clear, though colored; for there has been no precipitation of matters during drying which are afterwards hard to remove.

When the preparation is to be mounted in balsam, a drop of the culture is dried in the center of a cover glass, which is then placed for some minutes in dilute acetic acid to remove matter which would cause turbidity. The cover glass is then washed, dried, and stained for some hours in methyl violet.

I have made no attempt to study the life history of the nitrous organism, and the various forms which it assumes; by observations can only be regarded as furnishing material towards a more accurate knowledge of the subject.

The first form I have to mention consists of nearly spherical corpuscles, varying in size from mere points up to a diameter of 1.0μ, which extreme limit is, however, rarely reached. This form is very

striking, as it takes the stain deeply, and the extreme difference in size forms a remarkable picture. Occasionally the corpuscles of largest size are about to divide, and are seen as double organisms. This form is present in all cultures, and in some, as in milk and broth cultures 40 days old, was the only form observed. The minute point-like organisms certainly require explanation. They seem far too small to be produced by division of the full-sized organism.

The second form is the one generally described as the nitrifying organism. It is always longer than broad, and usually attains a length distinctly exceeding 1.0μ. It appears frequently as a nearly regular oval. Sometimes it is egg-shaped, one end being larger than the other. Sometimes the ends are more or less truncated, and occasionally sharply so. Not unfrequently the form is much less regular. This phase of the organism takes the stain more feebly than the circular form; the more irregular and broken (?) it becomes, the more feebly it is stained.

One would naturally conclude with Winogradsky that the darkly staining circular forms were the young organisms; but, as already mentioned, a difficulty arises from the extreme minuteness of many of them.

I have seen nothing similar to Frankland's thread-forming bacillus, which he found in broth cultures.

The first pair of photographs* shows the nitrous organism as it appeared in an ammonium carbonate solution, and upon the cover glass sunk for 70 days in the same solution: this culture contained a few bacilli. Both photographs show the large elongated form of the organism, and on the sunk cover glass the maximum size is seen. The same large form of the organism occurs in ammonium sulfate solutions.

The second pair of photographs shows the organism as found in weak broth, and in a 0.5 per cent milk solution. In both cases only the small circular form appears.

The magnification is in all cases 1,000 diameters.

The Nutrition of the Nitrous Organism

Independence of Organic Food

It has been hitherto held by vegetable physiologists that plants which are destitute of chlorophyll cells derive their carbon solely from organic matter, and are incapable of decomposing carbon dioxide. This doctrine apparently does not hold good in the case of the nitrifying organism, and possibly in that of some other bacteria.

It is curious that my first attempts at nitrification in solutions were made with no added organic matter, save the soil used as seed. Almost

*Not reproduced here. *Editor.*

immediately, however, in obedience to the received opinion, I commenced the practice of including a neutral tartrate among the constituents of my solutions. The advantages attending the presence of this tartrate were not very apparent. In 1879, I say, "A proportion of organic carbon to nitrogen equal to 3:10 by weight is sufficient for the purposes of nitrification, and probably a smaller proportion of carbon would suffice." In the next report (1884), I compare the rate of nitrification in solutions containing 40 mg. and 160 mg. of sodium potassium tartrate per liter. "The advantage as to speed of nitrification was in seven instances with the larger proportion of tartrate, in three instances with the smaller, and in five instances the rate of nitrification was quite similar." With glucose instead of tartrate, in the proportion of 20, 50, 100, and 200 mg. per liter, the advantage as to speed of nitrification was always with the smallest proportion of glucose.

In 1886, Dr. J. H. M. Munro raised the question: Is organic carbon essential to nitrification? He fully accepted the theory that organic matter is necessary for the nutrition of bacteria; he nevertheless records many experiments, made in the presence of mixed organisms, in which organic matter was plainly injurious to nitrification; and he has some experiments in which nitrification took place without any organic matter having been added to the solutions. He concludes that "the merest traces of organic matter, such as may be furnished accidentally by occasional exposure to the dust of the air, are sufficient for complete nitrous fermentation of the quantities of ammonia used in these experiments."

After the publication of Dr. Munro's results, I made a few experiments with the view of ascertaining whether the presence or absence of a tartrate had any influence on the progress of nitrification. The experiments were devised so as to test at the same time how far the so-called "incubation period," preceding nitrification, could be diminished by carrying out to a fuller extent the plan I had previously described, and with which Dr. Munro had been still more successful.

Three similar bottles each received 0.1 gm. of soil, 100 cc. of an ammoniacal solution, and some calcium carbonate. In bottle A, the solution merely contained 0.008 gm. of ammonium chloride. In AM, there were, in addition, potassium phosphate, and magnesium sulfate. In AMT, the solution contained, in addition to the constituents in AM, sodium potassium tartrate, equal in weight to the ammonium chloride. The experiment commenced on July 8th, 1886. As soon as any solution had nitrified, it was decanted off, and 100 cc. of a similar solution put in its place. The table on the following page shows the comparative rapidity with which nitrification began, and the time occupied in oxidizing the ammonia, in each solution.

The upper half of the table shows that when fresh ammoniacal solutions were placed in bottles in which nitrification had already occurred, the interval that elapsed before nitrification commenced

Interval before Nitrification Commenced

Solution	1st culture		2d culture		3d culture		4th culture	
	Days before starting	Order of starting	Days before starting	Order of starting	Days before starting	Order of starting	Days before starting	Order of starting
A	22	3	about 1	2	not 1	3	not 1	3
AM	13	2	about 1	1	not 1	1	not 1	1
AMT	9	1	about 1	2	not 1	2	not 1	2

Duration of Nitrification (days)

Solution	1st culture	2d culture	3d culture	4th culture
A	22	22	23	50
AM	24	10	9	19
AMT	22	19	11	28

suddenly diminished from many days to one, or less, a result perfectly accordant with that obtained by Dr. Munro. After the first culture, the solution AM, containing phosphates, etc., but no tartrate, was always the first to commence nitrification; but the difference between the three solutions is very trifling, amounting to only a few hours.

Again, after the first culture, the period of nitrification is always shortest in solution AM, and longest in A, where ammonium chloride alone was present. The tartrate has here a distinctly retarding effect on nitrification. It should, however, be mentioned that a larger proportion of nitrous acid, and less nitric, was found at the close of nitrification in AM than in AMT, so that the oxidation effected in the two solutions was more nearly alike than the figures would indicate. The great lengthening of the periods in the fourth culture is due to the commencement of winter temperatures.

A shorter series of experiments was also conducted with similar solutions in which no soil was present, but the solutions were all seeded with three drops of a nitrified culture. The results again showed, though not in a marked manner, that nitrification commenced first where no tartrate was present. The most striking result was, however, obtained with the solution which contained the ammonium salt alone; here nitrification in each case began, but never proceeded to more than a small extent. The reason was doubtless the absence of phosphates, which I have already shown are essential for nitrification.

In consequence of these results, I gave up the employment of tartrates as a constituent of ammoniacal solutions to be nitrified, and, as already mentioned, carried out a series of cultures in an inorganic solution when preparing for the isolation of the nitrifying organism. Professor P. F. Frankland has also employed an inorganic solution for the same purpose. His series of successive cultures is far longer than my own, and extends over more than two years; as, however, he employed cotton wool stoppers, it is possible that organic matter might thus be introduced; my own experiments were made in stoppered bottles.

It is quite clear that the facts as yet stated do not amount to any

proof that the growth and development of the nitrifying organism is independent of the presence of organic matter; no evidence has, in fact, been given that our solutions did not contain traces of organic matter, though none was intentionally added. To Winogradsky belongs the credit of establishing, in an unmistakable manner, the independence of the nitrifying organism of aid from organic matter.

Winogradsky ascertained, in the first place, that nitrification proceeded equally well when organic matter was rigorously excluded; he next determined, by a process of wet combustion, the amount of organic carbon produced from inorganic materials during nitrification; this portion of his work will be found in his second and third memoirs. He found the quantity of carbon assimilated during an energetic nitrification to be quite considerable. It appeared to bear a fairly constant relation of the amount of nitrogen oxidized, although the latter varied much in the experiments he quotes. For 1 part of carbon assimilated, 35.4 parts of nitrogen as ammonia were, on an average, oxidized to nitrous acid. Winogradsky suggests that the first step in the formation of organic matter may be the construction of an amide from ammonium carbonate, and he reminds us that even the animal body is capable of effecting such a change, urea being in this case produced.

The doctrine that a vegetable cell destitute of chlorophyll, and growing in the dark, should be capable of constructing organic matter from an inorganic carbonate, presents quite as many difficulties to the chemist as to the physiologist, as the action in question is one requiring a considerable absorption of energy. The action becomes, however, perfectly possible, if we may take into account the energy liberated at the same time by the oxidation of ammonia. If the nutrition of the organism and its oxidizing effect on ammonium carbonate take place simultaneously, the formation of organic matter from carbonic acid will not be opposed to the teachings of thermochemistry; as with an oxidation of 35 parts of nitrogen for an assimilation of 1 part of carbon, there is an abundant margin for an excess of heat development.

The energy produced by the oxidation of ammonia is not, of course, available as an explanation of the nutrition of the nonnitrifying organisms which accompany the nitrifying organisms in inorganic cultures. We must recollect, however, that under these circumstances the nonnitrifying organisms form, numerically, only a small minority of the total organisms present, and that we have little evidence of their capacity for growing alone in the absence of organic matter.

Influence of Carbonates and Acetates on Nitrification

After the publication of Winogradsky's results respecting the assimilation of carbon from carbonates, I made a few experiments to ascertain whether the presence of soluble acid carbonates exerted any

favorable influence on nitrification. In a previous paper, I have shown that disodium carbonate, except when added in small proportion, hinders nitrification; while monosodium carbonate may be added in much larger quantity without harmful result.

Five wide-mouthed, stoppered bottles were half-filled with a solution containing 0.30 gm. ammonium sulfate, 0.10 gm. potassium phosphate, 0.07 gm. magnesium sulfate, and a little calcium chloride per liter; some precipitated calcium carbonate was added to each bottle. After sterilizing, bottle 2 had 24 bubbles of carbon dioxide passed through the solution. Bottle 3 received monosodium carbonate at the rate of 1 gm. per liter. Bottle 4, disodium carbonate equivalent to the preceding. Bottle 5, calcium acetate, 0.25 gm. per liter. Bottle 1 had no addition. All were seeded with the pure nitrous organism from C3 on November 28th, 1890. The bottles were kept at 22° C.

Examined after 33 days, it was found that nitrification was completed in solutions 3 and 5, and was nearly completed in 2. Nitrification had commenced in 1, but was far from being completed. No nitrification whatever had occurred in 4. Examined again after 68 days, nitrification was completed in 1, but had not commenced in 4.

Thus, with the pure nitrifying organism we find the same hindrance to nitrification from disodium carbonate which had been previously observed in impure cultures. The point, however, of chief interest is the advantage derived from passing carbonic acid, from the addition of monosodium carbonate, and from the use of calcium acetate.

In comparative experiments in which bacteria are the active agents, no definite conclusion should be drawn from one series of experiments, as it is impossible to introduce the same number of bacteria in each experiment, and the rapidity of the action depends much on the number of organisms present. The trial was therefore repeated.

Five bottles received an ammoniacal solution as before. After sterilizing, about 10 cc. of carbon dioxide were passed into bottle 1. Bottles 2, 3, and 4 received monosodium carbonate at the rate of 4, 6, and 8 gm. per liter. In order to obtain accumulative effects, solution 1 was seeded from solution 2 of the previous series; solutions 2, 3, and 4 from solution 3; solution 5 from solution 5.

After 28 days, it was found that nitrification was completed in solution 5; it has made considerable progress in 2, less progress in 1, and had not commenced in 3 and 4. In 58 days, nitrification was completed in 1 and 2; it had commenced in 3, but not in 4.

The calcium acetate again appears as most favorable to nitrification; indeed, the nitrification in solution 5 is one of the most speedy I have noticed. The monosodium carbonate is also again superior to the carbonic acid, and it appears that as much as 4 gm. per liter may be introduced without injury; 6 gm. per liter were, however, plainly a great hindrance to nitrification, and with 8 gm., no nitrification took place.

The beneficial effect which followed the addition of carbonic acid, or monosodium carbonate, may of course be regarded as possibly due to the increase of available base in the solutions; to the greater development, in fact, of a condition known to be essential to nitrification. I do not believe, however, that this is the true explanation of the facts; for the disodium carbonate, even in small quantity, was a hindrance and not a help. And in a similar series of experiments, to be presently mentioned, in which nitrites were converted into nitrates, the same beneficial action of monosodium carbonate was observed, although here a salifiable base took no part in the reaction. I am disposed, therefore, to regard the presence of an excess of carbonic acid in a nitrifying solution as exercising a beneficial influence by aiding the nutrition of the organism; it acts, of course, in combination with ammonia.

On the influence of calcium acetate a third experiment was made, using 0.25 gm. per liter, and again the effect was distinctly to hasten nitrification. The calcium salt was employed in these experiments because it permitted the destruction of the acetic acid without increasing the alkalinity of the solution.

The action of the acetate is certainly interesting. In the presence of the mixed organisms of soil, an acetate, according to Munro, hinders nitrification by favoring the development of organisms reducing nitrates; when the nitrifying organism alone has possession of the field, it certainly aids nitrification. There is no contradiction in the statement that the nitrifying organism can assimilate carbon both from inorganic and organic sources; the same is true of green plants, which, while they decompose carbon dioxide, can also be fed with sugar.

Pages 515 to 522 are not included. In them, Warington reviews some of his earlier Rothamsted work on nitrate production and describes his attempts to isolate the "nitric organism." This section follows the same pattern as that given for his work on the "nitrous organism." Editor.

Summary of Results

1) The nitrification occurring in a mass of aerated soil, or produced by seeding with soil a cold, weak, ammoniacal solution, is purely nitric in character. If the ammoniacal solution is strong, or the temperature raised, a large quantity of nitrous acid is produced, which is finally converted into nitric acid. Soil readily converts a solution of nitrite into nitrate.

2) Pasture soil produces nitrites more readily than arable soil.

3) A clay subsoil, down to four feet from the surface, was found to produce nitrates.

4) In 1878 ammoniacal cultures were obtained (a third or fourth from one seeded with soil), yielding only nitrites, which remained

permanent. Subsequently other cultures were in the same condition.

5) Such nitrous cultures produce nitrites alone in solutions of ammonia, asparagine, urine, and milk. Their action is not altered by cultivation in shallow liquids with abundant aeration, or by a temperature of 30° C.

6) It is difficult to prove that no trace of nitric acid is produced by this nitrous agent, from the want of a sufficiently good analytical method. If a small quantity of nitrate is formed, it must be as a by-product, as the nitrous agent does not oxidize nitrites.

7) It was at first thought that the age of the culture was the factor which determined the loss of the power of producing nitrates, but this idea was negatived by subsequent experiments. Successive cultivations in an alkaline solution (as ammonium carbonate) were found to be a certain method of obtaining the purely nitrous agent.

8) The production of nitrites by the above-mentioned agent is not due to a process of reduction; it readily takes place in inorganic ammoniacal solutions.

9) After the nitrification of an ammoniacal solution, if not too weak, gelatinous flocks frequently appear at the bottom of the vessel. If precipitated calcium carbonate has been introduced, it assumes a curdled appearance; and when treated with acid, the gelatinous matter is left. When highly magnified, the jelly is seen to consist of highly refractive, circular corpuscles.

10) A large number of organisms, from many sources, obtained as pure cultures on gelatin, were introduced into ammoniacal solutions, but no nitrification was in any case obtained.

11) Successive cultivations, the first seeded with soil, were made in an inorganic ammonium carbonate solution, supplied with phosphates, etc.; the product became wholly nitrous at the second culture.

12) The organisms seen in these ammonium carbonate cultures were chiefly cocci, oval or round, the latter the smaller, and staining most deeply.

13) When such ammoniacal cultures were spread on gelatin, or on agar-agar, no such cocci appeared, although the composition of these media had been specially contrived to agree with that of well-known nitrifiable solutions. From several of the ammoniacal cultures only a single species of bacillus was obtained by cultivation upon gelatin or agar-agar.

14) None of the growths obtained on gelatin from nitrified solutions were found to produce nitrification when introduced into suitable liquid media, nor when placed on marble moistened with an ammoniacal solution.

15) Broth, and broth with solution of ammonium carbonate, were seeded with the nitrified cultures and examined microscopically. The bacilli present in the cultures used as seeds were greatly developed in the broth; besides these, only small, dark points were seen in the

stained preparations. The mixture of broth and ammonium carbonate readily nitrified.

16) Three series of trials to separate the nitrifying organism by the dilution method failed; a fourth, in which an ammonium chloride solution with calcium carbonate was the culture liquid, succeeded. Out of twenty solutions, ten nitrified, and of these three gave no growth on gelatin.

17) The organism thus separated oxidizes ammonia to nitrous acid only; it is, in fact, the nitrous agent studied in my earlier experiments. Solutions of a nitrite seeded with this organism do not yield nitrate.

18) The nitrous organism grown in broth containing calcium nitrate did not reduce the nitrate to nitrite.

19) The nitrous organism does not grow on gelatin, or on agar-agar. It grows slowly in weak broth, but without impairing its transparency, or producing any other visible change.

20) The pure nitrous organism is capable of producing nitrous acid in solutions of asparagine, milk, urine, and urea; the last-mentioned was apparently the most difficult to attack. The nitrification of the milk and asparagine made slow progress. All the cultivations of the pure organism were free from turbidity.

21) The nitrous organism occurs as nearly circular corpuscles, varying from minute points up to nearly 1.0μ in diameter, sometimes double when division is approaching; these circular organisms stain deeply. It may also occur as oval cocci, the lengths frequently exceeding 1.0μ, and the ends not unfrequently more or less truncated. There may also occur very irregular and broken (?) forms which take stain but feebly.

22) When the mixed organisms of soil are present in an ammoniacal solution, nitrification is hindered by the presence of a tartrate.

23) The nitrous organism will readily develop, and oxidize ammonia to nitrous acid, for apparently an unlimited number of successive generations in solutions to which no organic matter has been added: but phosphates are essential for this development. Winogradsky has supplied quantitative proof that the organism produces organic matter from inorganic materials, and finds on an average about 35 parts of nitrogen oxidized for 1 part of carbon assimilated from ammonium carbonate. The energy developed by the oxidation supplies apparently an explanation of this otherwise improbable reaction.

24) In pure cultures of the nitrous organism in ammoniacal solutions, the addition of carbonic acid, monosodium carbonate, or calcium acetate facilitates nitrification, the addition probably supplying carbonaceous food. Disodium carbonate greatly hinders nitrification.

25) Results obtained in 1880 and 1881 revealed the existence of an organism which energetically converted nitrites into nitrates, but was apparently unable to oxidize ammonia.

26) In 1886 and 1890, attempts were made to separate the active

organism from the 1881 cultures by growth on gelatin and potato; none of the organisms thus separated had any power of oxidizing ammonia or nitrates.

27) Recent results show that the nitric organism develops in inorganic solutions, and energetically converts nitrites into nitrates, especially if supercarbonates are present. Monosodium carbonate, 1 to 4 gm. per liter, exerted a favorable influence, 6 gm. a retarding influence. Disodium carbonate greatly hinders its action.

28) The nitric organism produces neither nitrites nor nitrates in ammoniacal solutions, even when carbonic acid, or monosodium carbonate, or calcium acetate is supplied.

29) The presence of ammonia is apparently a hindrance to the action of the nitric organism on nitrites, even when monosodium carbonate is present.

30) The dislike of the nitric organism for ammonia explains the course of nitrification when comparatively strong solutions of ammonium salt (1 gm. per liter) are seeded with a small quantity of soil; much nitrous acid is then produced, and the formation of nitric acid sets in only when the ammonia has greatly diminished in quantity.

31) An attempt to isolate the nitric organism by the dilution method failed; but apparently only one other organism, a stout bacillus, growing on gelatin, was present in some of the cultures. The stained preparations from these cultures contained an abundance of the minute circular organisms observed in pure cultures of the nitrous organism; the form of the two organisms is thus apparently similar.

32) The nitrification effected by soil is thus explained as performed by two organisms, one of which oxidizes ammonia to nitrites, while the other oxidizes nitrites to nitrates. The first organism is easily separated from the second by successive cultivations in solution of ammonium carbonate. The second is (probably) separated as easily from the first by successive cultivations in solution of potassium nitrite containing monosodium carbonate.

33) In soil, the nitric organism is equally active as the nitrous, since soil never contains any but extremely weak solutions of ammonia, and supercarbonates are always present.

Further Reading

A. D. H. "Robert Warington, F. R. S." *Nature*, LXXV (1907), 511-512.

P. S. U. P. "Robert Warington, 1838-1907." *Proceedings of the Royal Society of London*, Series B, LXXX (1908), xv-xxiv.

XV.

MARTINUS WILLEM BEIJERINCK

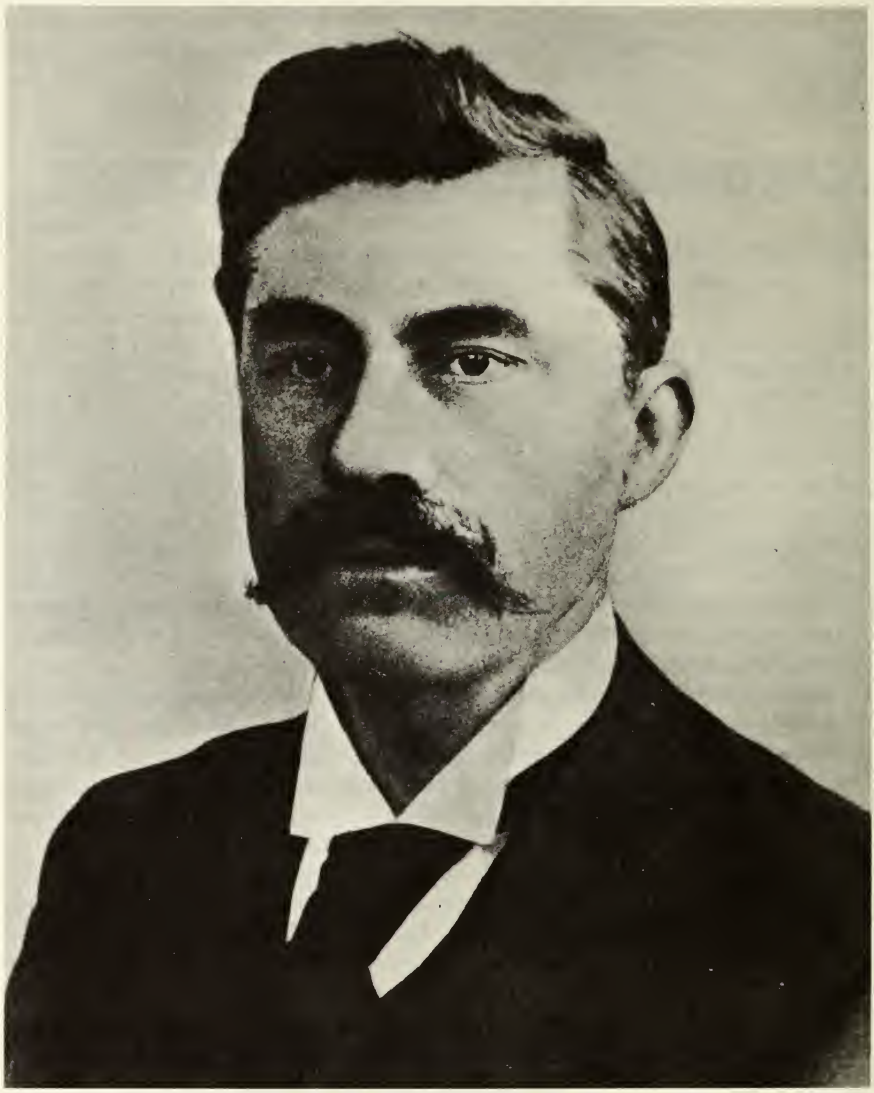
(1851 to 1931)

Martinus Willem Beijerinck was born in Amsterdam, Netherlands, on March 16th, 1851. He attended the Polytechnical school at Delft from 1869 to 1872, and during this period became well acquainted with J. H. van't Hoff, the great physical chemist. In 1872 he entered the University of Leiden, and after completing preliminary work, obtained a teaching post at the agricultural school at Waffrum, in the province of Groningen. Beijerinck earned his doctorate from the University of Leiden in 1877 with a dissertation on plant galls. During the next few years (1876 to 1884), while teaching at the agricultural high school at Wageningen, he devoted himself to botanical studies, a number of which were later published.

Beijerinck was elected a member of the Royal Academy of Sciences at Amsterdam in 1884. In 1885 he was employed as a bacteriologist in the Nederlandsche Gist-en Spiritusfabriek in Delft. He was attracted to this position by excellent salary and a new, well-equipped laboratory. Ten years later he entered on his university career as professor of botany at the Polytechnical School at Delft.

Subsequently, Beijerinck was made a Knight of the Order of the Nederlandsche Leeuw (1903), an honorary member of the Royal Botanical Society of Edinburgh (1906), and a Commander of the Order of Orange-Nassau (1921). In 1926 he was elected a Foreign Member of the Royal Society of London, a reminder of Leeuwenhoek's election over two hundred years earlier. Beijerinck died on January 1st, 1931, at Gorssel, Netherlands.

During his long career, Beijerinck and his students made a number of highly significant contributions to microbiology. Among them were isolation of the nitrogen-fixing bacterium *Bacillus radicicola (Rhizobium leguminosarum);* elucidation of oxygen relationships among bacteria ("respiratory figures"); extension of ideas concerning the physiology of luminous bacteria; use of the elective culture method and development of the auxanographic technique; and extension of



Martinus Nijhoff, The Hague

Martinus Willem Beijerinck

fundamental knowledge concerning yeast enzymes, lactic acid, acetic and butyl bacteria, viruses, denitrification, urea hydrolysis, and microbial variation.

One of Beijerinck's most important contributions to microbiology was the utilization of the principle of enrichment cultures. It was by the use of this method that the major transformations of matter brought about by specific bacteria were revealed. In addition, the relationship between environmental circumstances and specific types of bacteria thriving under them led to the "ecological approach" to microbiology.

In Beijerinck's words (1905): "In an experimental sense the ecological approach to microbiology consists of two complementary phases which give rise to an endless number of experiments. On the one hand, it leads to investigating the conditions for the development of organisms that have for some reason or other, perhaps fortuitously, come to our attention; on the other hand, to the discovery of living organisms that appear under predetermined conditions, either because they alone can develop, or because they are the more fit and win out over their competitors. This latter method, in reality nothing but the broadest application of the elective culture method, is especially fruitful and truly scientific; and it is no exaggeration to claim that the rapid and surprising advances in general microbiology are due to this methodology." (In van Niel, 1949.)

Beijerinck presented a preliminary paper in 1894 in which he described *Spirillum desulfuricans* (*Desulfovibrio desulfuricans*), in detail, as the causative organism of sulfate reduction. His interest in this problem was quite practical, for at this time he was employed at the Nederlandsche Gist-en Spiritusfabriek, and the yeast factory was confronted with the problem of freeing the canal water used in the steam boilers from the calcium sulfate present therein. It is typical of Beijerinck that he sought a biological means of accomplishing this task.

The following is a translation of Beijerinck's three 1895 publications, entitled "Uber *Spirillum desulfuricans* als Ursache von Sulfat-Reduction." The first of these was the first paper published in the new Section 2 of the *Centralblatt für Bakteriologie*, I (1895), 1-9; 49-59; 104-114.

ON *SPIRILLUM DESULFURICANS** AS THE CAUSE OF SULFATE REDUCTION

The formation of hydrogen sulfide and other sulfides by living organisms is a natural phenomenon of general occurrence, and is important from a purely scientific point of view, as well as on geological and hygienic grounds. In biological respects, the importance of this process lies in the rather extensive flora and fauna adapted specifically to the production of hydrogen sulfide; they include infusoria, flagellates, bacteria, and many green or otherwise pigmented algae.[1] The geological significance is obvious from the observation that the mud of entire seas and many lakes is rich in iron sulfide. Thus the floor of the Black Sea is covered with an enormous layer of iron sulfide-containing mud, above which the water contains hydrogen sulfide to a considerable height. At one depth of 2,125 meters no less than 6.5 cc. of hydrogen sulfide per liter were found. The greatest portion of this sulfur compound resulted from the reduction of sulfate, but a small quantity was carried over from sulfur-containing proteins.

H. Braconnot was the first to observe the occurrence of iron sulfide in black sewer sludge and in the subsoil of Nancy, and he believed that there was a connection between it and the spread of cholera. Since then it has been recognized that this material is found in clay soil and in the alternately moist and dry seashores, as well as in the mud of swamps, ponds, and streams, and that its presence is, in general, governed by living organisms; yet only scant data are found in the literature. It has been found that the ground water in deep soil strata, at least in south Holland, is completely, and in the province of Gelderland nearly completely, sulfuric acid-free, and the question was raised whether microbes from surface waters carried out a reduction of the sulfate in deep-percolated ground waters.

If this is indeed the case, the following reactions should occur in such water: (1) At the upper strata, but only where a complete oxygen deficiency prevails (the sulfide ferment is, as we shall see later, strongly anaerobic), hydrogen sulfide is formed from sulfates. (2) In the same vicinity, iron sulfide is produced from ferrous or ferric compounds; in the case of ferric salts the precipitation is influenced by the presence of reguline sulfur. (3) Decomposition of iron sulfide by carbonic acid with hydrogen sulfide formation. (4) Oxidation near the surface of iron sulfide or hydrogen sulfide to sulfate or sulfur.

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*This organism is now classified as *Desulfovibrio desulfuricans*. *Editor*.

[1]A flora and fauna adapted to hydrogen sulfide is found in brackish and sea water, and we do not ordinarily find these organisms in sweet water. See E. Warming, *Danmarks Kyster-Bakterier*, Copenhagen, 1879.

(5) Formation of hydrogen sulfide or sulfuric acid from the resulting free sulfur. Those compounds which diffuse into the deep soil strata include calcium and ferrous carbonate; and if sulfate reducing organisms are present, sulfur compounds are not far removed from these in the underlying layers. The reason for the presence of sulfur near the surface in these transformations is that the diffusion of hydrogen sulfide and other sulfides leads to a continuous oxidation here by free oxygen; that is, there is a diffusion to a hydrogen sulfide-free area, and in deeper layers the diffusion is prevented by iron sulfide formation. Carbonic acid forms soluble salts in the deep soil layers, and these penetrate still deeper into the earth.

According to this theory, there must be areas in the soil where the sulfide ferment cannot live, for example, where oxygen is present or where there is no organic matter at all, and sulfuric acid is therefore not formed.

In the places cited above, iron sulfide appears either as an insoluble salt Fe_2S_3 or as hydrates. The hydrated sulfide may be insoluble or occur as the water-soluble, green-black form. Regarding this, A. Gautier has stated that pyrite (FeS_2) is formed in marshes through oxidation of iron sulfide or iron carbonate in the presence of free hydrogen sulfide.[2] Perhaps this should be a sixth reaction added to the five mentioned above as occurring in the earth's surface. At any rate, the question of the distribution of biologically formed pyrites is one of considerable interest.

In general, one finds little pure sulfur in the soil; ordinarily it is in an unstable state in the vicinity of water wherein bacteria are found, and through the latter this element is transformed to hydrogen sulfide. This occurs most readily under alkaline conditions, a reaction brought about by many bacteria. In each instance I am convinced that finely divided sulfur results from hydrogen sulfide oxidation, and as readily from action by dilute ammonia as from contact with bacteria living in water; but in the absence of air, a black iron salt of sulfide is produced.

The Various Modes of Biological Formation of Hydrogen Sulfide

The modes of formation of hydrogen sulfide by microorganisms are as follows: first, by the transformation of sulfur-containing proteins; second, directly from reguline sulfur; third, from sulfite and thiosulfate, in which the thiosulfate is previously split to sulfur and sulfide; and fourth, by sulfate reduction.

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[2] Pyrite precipitation from coal tar liquor may be due to this, and thus ultimately to our sulfide ferment.

In the case of the first two, hydrogen sulfide formation from protein and sulfur may occur without microbial mediation. Thiosulfate may be included here too, but the chemistry of this has not been investigated as far as I know. Only sulfate reduction appears dependent on bacterial action, and it is the only instance on which a biological agency must be involved.

In regard to hydrogen sulfide formation directly from proteinaceous material, it will be recalled that simply by boiling there is developed as much as 0.1 per cent hydrogen sulfide, and that in the wort of breweries and compressed yeast factories, in the absence of microbial activity, boiling in the presence of traces of carbonic acid gives rise to soluble sulfide.

The transformation of sulfur, through contact with easily altered organic materials, to hydrogen sulfide has been the subject of several investigations. Blood, egg albumin, egg yolk, and yeast extract were examined in this regard, as well as the fluids of various animal organs. It is doubtful, however, that a satisfactory differentiation can be made between sulfide from sulfur, and sulfide from organic compounds. Boehm stated that spring water plus flowers of sulfur developed hydrogen sulfide when sealed in flasks under anaerobic conditions, whereas pure water did not do this. J. Rey-Pailhade has presented data regarding yeast extract in which he finds that the hydrogen sulfide-engendering material (which he terms "Philothion") is soluble in dilute alcohol; and this extract, similar to wort, takes up oxygen and gives off carbon dioxide during its decomposition. In order to produce active extracts, the yeast cells must be ground and extracted with cold water. The activity is lost upon treatment with charcoal, and it depends, apparently, on a coagulable protein.

The easiest way of showing the transformation of sulfur to hydrogen sulfide by mediation of a living agency is by adding flowers of sulfur to a strong-smelling water sample or to a sugar solution vigorously fermented by yeasts. A piece of lead acetate paper exposed to the atmosphere above such mixtures becomes deep brown in a few minutes. Since this occurs with pure cultures of yeasts in sucrose solution, it is evident that hydrogen sulfide formation is not an exclusive ability of bacteria.

Since there exists uncertainty concerning the chemistry of this reaction, perhaps it is to be explained upon the basis of action of yeasts on some diffusible materials identical to the active principal of Rey-Pailhade.

The following two experiments will serve as a demonstration of the biological formation of hydrogen sulfide in the presence of proteinaceous material.

One fills a 25-cc. Kuhne fermentation flask with oxygen-free meat broth (obtained by boiling) and 0.1 per cent ferrous lactate or Mohr's salt (ferrous ammonium sulfate) as indicator; a second flask is

prepared in the same manner but with the addition of flowers of sulfur. Each flask is then inoculated with a drop of ditch water or some garden soil and incubated at 30°C. Sulfate reduction does not take place in these flasks. After 24 hours a blackening due to iron sulfide formation begins to develop. It should be noted that in the flask without added sulfur the darkening quickly attains its maximum, whereas in the other flask it progresses to the point where a deep black precipitate develops.

Hydrogen sulfide formation from thiosulfate is readily demonstrated when the latter is added to a culture of growing yeasts. I added 0.1 per cent sodium thiosulfate ($Na_2S_2O_3 + 5H_2O$) to a flask of wort-gelatin medium, and to the still-liquid gelatin a pinch of pure yeast was introduced and the mixture allowed to solidify. A piece of lead acetate paper is suspended in the neck of the flask. After one day at room temperature one notices that the yeasts have grown vigorously and an intensive formation of lead sulfide has occurred.

During fermentations, the reducing ability of yeasts on thiosulfate is easily demonstrated. If one takes, for example, a 20 per cent sucrose solution in tap water to which 15 per cent fresh yeast is added in the presence of 0.05 per cent sodium thiosulfate, a complete decomposition of this salt to hydrogen sulfide may be shown.

Sulfite will also be decomposed to hydrogen sulfide under similar conditions. For example, 0.066 per cent sodium sulfite ($Na_2SO_3 + 7H_2O$) will quickly be changed to an equivalent amount of hydrogen sulfide. These processes are significant, since nitrate, nitrite, indigo-carmine, and litmus are not reduced by yeasts.[3]

Holschewnikoff's *Bacterium sulfureum* also decomposes sodium thiosulfate with hydrogen sulfide formation.

In this general discussion I should like to bring out several points from a little-known paper by N. D. Zelinsky.

This investigator described an organism, *Bacterium hydro-sulfureum ponticum* (*Desulfovibrio desulfuricans*), which formed hydrogen sulfide from the following: 1 per cent ammonium tartrate, 1 per cent fructose, 0.33 per cent sodium thiosulfate, 0.1 per cent calcium phosphate, and a trace of calcium chloride. It was reported, but without detail, that this organism could decompose sulfite and sulfate, and that aerobic or anaerobic conditions were satisfactory. A statement as to how, in the presence of other sulfur sources, the quantitative disappearance of a given amount of sulfite or sulfate, or the formation of hydrogen sulfide was measured, is not given in the abstract. In regard to the latter, the older works known to me on hydrogen sulfide formation from sulfate are not sufficiently conclusive.

I consider it important to review briefly the biological modes of sulfide formation, for in studies on sulfate reduction, wherein sulfide

[3]On the other hand, iodate is reduced by yeasts to iodide.

detection is desired, one must be aware that the merest tract of certain compounds may serve as powerful reagents, and therefore one must realize that there are other sources of sulfide besides sulfate.

Theories of Biological Hydrogen Sulfide Formation

Theoretical considerations of various authors regarding the chemical explanations for hydrogen sulfide formation by microorganisms are too numerous to review exhaustively, and, no less important, we are completely ignorant of the principal agents of sulfate reduction in our waters.

A few of the theories proposed by several well-known investigators will be summarized in the following review.

In many of these theories it is stated that the process involves the action of nascent hydrogen produced by microbes. R. S. Petri and A. Maassen have proved this chemically by showing that palladium black mixed with anhydrous flowers of sulfur, as well as solutions of thiosulfate, protein, or peptone at 50°C., generate hydrogen sulfide if a stream of hydrogen is passed through them so that the atmosphere is excluded. In addition, these authors presented evidence that hydrogen sulfide is produced from ammonium sulfate when acted upon by hydrogen generated through the action of hydrochloric acid on zinc. I attempted to repeat this experiment, using pure, sulfur-free zinc in an air-free 5 per cent ammonium sulfate solution, but could not demonstrate hydrogen sulfide in this or in more dilute solutions. I can only conclude that these investigators used very concentrated solutions of ammonium sulfate and hydrochloric acid (I presume sulfur-free zinc was used), and they therefore produced a strong solution of sulfuric acid, which gives sulfur acids, and these reacted with hydrogen to produce hydrogen sulfide; this reaction is quite different from the biological process we are considering here.

I do not believe that chemical analogies necessarily duplicate physiological processes. If this idea were to be entertained, it would be necessary to demonstrate hydrogen in the presence of the reducing organisms, and it is certain that with the sulfide ferment described here no hydrogen is produced, and this is equally true in the case of yeasts.[4] On the other hand, ability to produce hydrogen sulfide from sulfate is absent in bacteria which produce hydrogen. Organisms such as coli bacteria or anaerobic granulobacteria (such as the butyl ferment, *Gr. butylicum* [*Clostridium butyricum*], and *G. lactobutyricum*) that rapidly reduce indigo-carmine and litmus, are notable examples.

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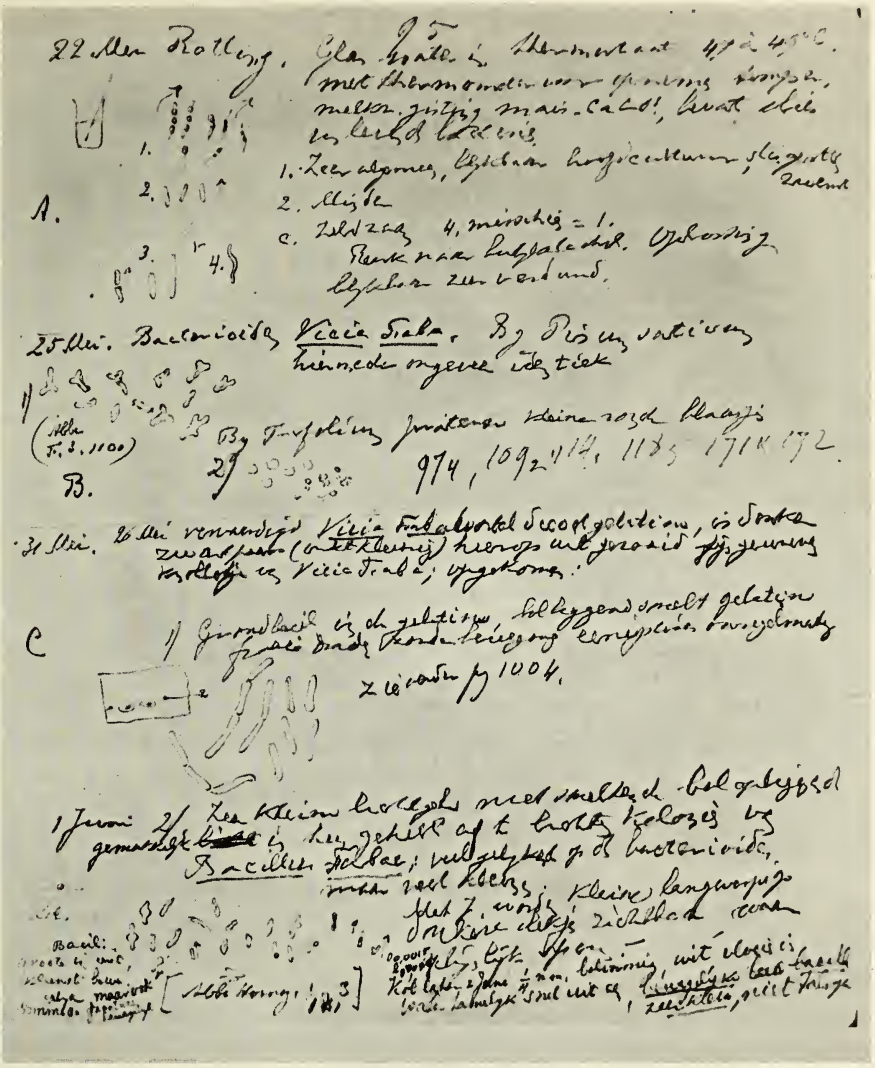
[4]Hydrogen ions are generally found in the presence of yeast cells; if this were the reason for the reduction, every acid would be a reducing medium.



Martinus Nijhoff, The Hague

A page from Beijerinck's laboratory notebook

E. F. I. Hoppe-Seyler has attempted to relate this process to the methane fermentation of cellulose, which proceeds differently in the presence of gypsum and iron oxide than in their absence, and generates hydrogen sulfide only with the aid of iron sulfide and calcium carbonate. Hoppe-Seyler, therefore, does not consider sulfate reduction as an "independent process carried out through lower organisms." He apparently was not convinced by his experiments that the process was a biological one, as against other possible explanations. In my view, the process is most certainly biological; and in the sections that follow, proof of this will be given. It will be shown that the reduction of calcium sulfate with hydrogen sulfide formation is brought about by a specific agent, and that in any event it is not directly connected with either methane formation or cellulose fermentation. In Hoppe-Seyler's work, filter paper was attacked in the presence of sewage sludge, and methane and carbon dioxide were formed. If it is assumed that during the course of the fermentation the filter paper was digested, it is then conceivable that many other microorganisms, including the problematic methane ferment, could grow upon it.

We can now explain why the ratio between carbon dioxide and methane, which was found under normal experimental conditions to be 1:10, could be altered by the presence of gypsum (and iron oxide) by allowing the development of sulfide-producers and numerous other microbes and thereby become 10:1. It is true that this explanation assumes the presence of cellulose-splitting enzymes in the methane fermentation, and at the present time this enzyme is an unknown as the methane ferment itself. In all of these explanations one encounters uncertainties, and it seems not premature to propose a new theory. This will come closer to the basic causes underlying the process, now so little understood.

The relatively crude methods used led to the discovery of the sulfide ferment, and from this, experimental data supporting this theory were obtained.

In reference to the direct transformation of reguline sulfur to sulfide, as pointed out in the introductory part of this paper, this is related, in my opinion, to alkali formation, generally brought about by certain bacteria.

Quantitative Estimation of Products of Sulfate Reduction

In this section the methods for the chemical determination of sulfate reduction products are given, together with methods of standardization, etc. Editor.

Sulfate Reduction in Water and in Liquid Media by Crude Cultures of the Sulfide Ferment[5]

It is not difficult to demonstrate complete reduction of sulfate in water, or in a diluted, nonsterile liquid medium. In nature, for example in drainage canals, one finds, particularly in summer, a nearly complete disappearance of sulfate due to pollution of the water with sewage, and there is a strong stimulation of the reduction process with hydrogen sulfide formation. In many cities in Holland the situation has become quite bad, and only a complete and costly water treatment plant will bring about an improvement. During the reduction process the oxygen content of the water decreases to nil, or to state it another way, the oxygen is completely consumed due to the activities of the bacteria in the water in attacking the organic matter therein, and thereafter sulfate reduction begins in earnest. Then the fish die, so it is understandable that under these circumstances there is a complete transformation of the microscopic fauna. Certain infusoria increase extraordinarily; and if one allows a container of this kind of water to stand, its surface is shortly completely covered with a dense film of these oxygen-requiring organisms.[6]

What nature carries out on a large scale, one may duplicate in the laboratory on a small scale, and indeed very instructive experiments such as the following, were performed:

The sulfide ferment is especially plentiful in canal water during the months of July, August, and September; later in the year it is less abundant or entirely absent. I have never failed to find it in sewage sludge, and even a very small bit of black mud from our inland waters constitutes an unfailing inoculum for conducting reduction experiments. The sulfide ferment may be found in garden soil to a depth of 5 cm., but I have not sought it in deeper layers. Apparently it can occur in them.

In the above-mentioned months, one needs to introduce only a small amount of organic matter in order to bring about a fully operative sulfate reduction, indeed only 12 to 24 hours at 25° to 30°C. are needed. Three conditions must be met: Exposure to oxygen must be prevented; the added organic matter must not give rise to acid formation, and therefore the carbohydrate content must be minimal so that the water bacteria will quickly convert it to carbon dioxide and water; and phos-

[5]By "crude cultures of the sulfide ferment" I mean the bacterial mixture present in natural waters, in which all the necessary conditions for the development of the sulfide ferment are met.

[6]I have not sought explanations for the presence of such great numbers of infusoria, but they require a definite, though low, concentration of dissolved oxygen and are similar in this requirement to "spirilla types."

phate and other salts ought to be present—nitrogen compounds need be added only if a reduction greater than 60 mg. of SO_3* per liter of water is expected—otherwise canal or sewage water will contain enough to cover the requirements of the sulfide microbes. At the start of this investigation, which involves the isolation of the sulfide ferment from material containing large amounts of it, I had to depend on canal water, for often it is not found elsewhere. It was never found on the sides of boats, or in the dust of stables, or in the atmosphere.[7]

Sulfate reduction occurs best in very dilute nutrient solutions. The sulfide ferment does not liquefy gelatin and forms little acid, which perhaps would attack agar, but neither gelatin nor agar inhibits sulfate reduction. The ferment is not sensitive to soluble organic compounds, as is the nitrite ferment of ammonium salts, which, although it may strongly nitrify in the presence of exhaustively washed agar, is only weakly active in the presence of gelatin treated similarly.[8] It should be observed that the end-products of bacterial activity are not deleterious to the first-mentioned ferment; in fact, they may aid it, and in this regard the earlier cited favorable influence of natural canal water should be kept in mind. In any case, the ferments grow better in bacterial mixtures than in pure cultures.

In agreement with later observations, liquid cultures appeared very clearly to carry out strong reduction reactions. There occurs mainly in soil an inorganic mud, formed under the influence of sodium carbonate. This deposit never fails to yield the sulfide ferment; for in it, anaerobic conditions develop readily, In my studies, the mud used consisted principally of calcium phosphate and carbonate, together with the organic matter of canal water. Iron phosphate and carbonate also serve as substrates for the ferment. In iron-containing mud one finds a blackening indicative of reduction; this occurs first as narrowly circumscribed spots which then gradually expand until the entire mass is deep black. Since the sulfide ferment generally occurs in locations in which it is actively motile, it must be made non-motile if exceptional development is to be seen. I have observed that under good nutritional conditions, motile bacteria are usually at rest.

The aggregation of the sulfide ferment in inorganic mud has analogies with the nitrification reaction, but there is also a significant difference in that the chalk mud in which the nitrite ferment is found must be saturated with oxygen, whereas the sulfide ferment thrives in the complete absence of this gas.

Addition of the ferment to a liquid medium containing a definite amount of sulfate results in the formation of hydrogen sulfide, and this is brought about in several different ways. The simplest, which usually is successful, is as follows:

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*Now written as SO_4. See note 13. *Editor*.

[7]The sulfide ferment is apparently killed by drying.

[8]One does not find a distinct gelatin liquefaction in the case of the nitrite ferment of ammonium salts.

To 1 liter of canal water are added 3 cc. of a malt wort of 10° Balling, 1 gm. of crystalline sodium carbonate ($Na_2CO_3 + 10H_2O$), and 0.2 gm. of Mohr's salt ($FeSO_4 + (NH_4)_2SO_4 + 6H_2O$). A slowly settling precipitate of calcium phosphate and ferrous carbonate is formed. If the sulfuric acid content of canal water is 40 mg. per liter, the solution therefore contains 40 + 81.6 = 121.6 mg., which, due to the 28 mg. of added iron, may bind 66 mg. as iron sulfide, and 55.6 mg. will be bound as calcium sulfide or some other sulfide; thus reduction will be complete. One shakes the mixture vigorously so that the precipitate is homogeneous, and then fills a bottle to the stopper, or else a glass beaker covered with a flat glass plate in such a way that the entrance of air is prevented. One then incubates the mixture at not above 25° to 30°C. It is true that the reduction can be carried out as high as 40°C., but the optimum temperature for the reaction is 25°C. or slightly below. In cases wherein a temperature higher than 25° to 30°C. is found better, other bacteria besides the sulfide bacteria grow; and because of their oxygen requirements and other physiological properties, such as through alkali or acid formation, they exert an important influence on the reduction reaction. With regard to the lower temperature limits, I have found that, at least in enrichment cultures, reduction is possible down to 12°C.; however, below 20°C. the reaction is uncertain or does not occur; the temperature should not fall below 20°C.

The experiments cited here were conducted in 2-liter flasks, completely filled and glass stoppered. Each day 25 cc. of the contents were titrated and the flasks again filled with the original mixture.

The course of the reduction reaction is seen in Table I.

At the beginning of this experiment there were present 121 mg. of SO_3 per liter, and from this 84 mg. of hydrogen sulfide were recovered, while after five days the mixture was shown to be completely free of sulfuric acid as indicated by the barium reaction; for 121 - 84 = 37 mg. SO_3, which must have been used in another way, either bound

Table I

Days after Start of Experiment	Cc. Normal Iodine per liter	Expressed as H_2S mg. per liter	Expressed as H_2SO_4 mg. per liter	Remarks
2	0.75	12.75	30	
3	1.5	25.5	60	
4	1.9	32.3	76	
5	2.1	35.0	84	No more H_2SO_4
6	2.1	35.0	84	Trace H_2SO_4

as organic matter, or as free sulfur, or to some other reduction product.[9]

After six days a trace of sulfuric acid was found, and hence it follows that the sulfate in 25 cc. of mixture added at the fifth day of the experiment was not completely reduced in 24 hours.

In another experiment a mixture was made as follows:

Canal water was saturated with gypsum ($CaSO_4 + 2H_2O$). About 2 gm. of gypsum dissolve in 1 liter of water. The solution contained approximately 1,000 mg. of SO_3 per liter. To each liter were added 50 mg. of sodium malate, 50 mg. of asparagin, 100 mg. of calcium phosphate, and 1 gm. of sodium carbonate; no iron salts were added. The course of the reaction is given in Table II.

Table II

Days after Start of Experiment	Cc. Normal Iodine per liter	Mg. H per liter*	per liter†	Remarks
6	1.5	25.5	60	
11	2.0	34.0	80	
13	2.5	42.5	100	Some sulfur deposition
15	2.9	49.3	116	
16	3.0	51.0	120	
18	3.5	59.5	140	
20	3.5	59.5	140	
21	3.1	52.7	124	Air entered, sulfur deposited

*One cc. of normal iodine equals 40 mg. of SO_3.

†Further experiments using completely different media and SO_3 concentrations gave such good results that I began to believe a good quantitative method for sulfuric acid determination had been found. Later work has shown that large variations may occur for reasons not yet clear, so that each determination includes some error.

A saturated gypsum solution with the essential organic nutrients is therefore a satisfactory medium for reduction experiments. Nevertheless, the reduction proceeded slowly in this experiment, and of the 1,000 mg. of SO_3, only 124 were reduced. As fresh medium was added, for some unknown reason reduction failed to proceed. The failure was not due to an accumulation of hydrogen sulfide, as is shown in the following experiment:

To canal water containing 37.5 mg. of SO_3 per liter were added, per liter, 130.6 mg. of Mohr's salt ($FeSO_4 + (NH_4)_2 SO_4 + 6H_2O$), con-

[9]One cc. of normal iodine solution equals 17 mg. of hydrogen sulfide.

taining the equivalent of 53.3 mg. of SO_3, 492.6 mg. of $MgSO_4 + 7H_2O$, SO_3 equivalent to 160 mg., 100 mg. of NaCl, 100 mg. of sodium malate, 100 mg. of asparagin, 200 mg. of calcium phosphate, and 1 gm. of sodium carbonate. The mixture contained a total of 37.5 + 53.3 + 160 = 250.8 mg. of SO_3 per liter.[10]

The reaction proceeded very rapidly, as shown in Table III.

Table III

Days after Start of Experiment	Cc. Normal Iodine per liter	Expressed as mg. H_2S per liter	Expressed as mg. SO_3 per liter	Remarks
3	1.6	27.2	64	Entire liquid black
4	2.0	34.0	80	Iron sulfide precipitates
5	2.3	39.1	92	Liquid clear
6	2.8	47.6	112	
7	3.4	57.8	136	Some sulfur deposited on glass
8	3.6	61.2	144	
9	3.7	62.9	148	
15	4.1	69.7	164	H_2SO_4 disappeared
20	3.0	51.0	120	Air entered, much sulfur

Of the 250.8 mg. of SO_3 initially present, 164 mg. were recovered in hydrogen sulfide, and 86.8 mg. were changed in other ways, partly to sulfur.

Up to this point I had performed no experiment in which a greater concentration of hydrogen sulfide, namely nearly 70 mg. per liter, accumulated, and above which the compound would be inhibitory to the sulfide ferment. If 1 cc. of hydrogen sulfide gas weighs 1.5 mg., these results are equivalent to 46 cc. per liter.[11]

The small amount of sulfuric acid found in Delft canal water is quickly reduced when one adds to it an organic nutrient, such as sucrose, starch, glycerin, asparagin, lactate, tartrate, or peptone, together with calcium phosphate, iron chloride, and sodium carbonate.

[10]Here one can see that salt was added. Later experiments showed that 3 per cent salt used in my earlier work indeed inhibited enrichment cultures of *Spirillum desulfuricans*, but the process was stimulated when the amount used was reduced.

[11]Bakhius Roozeboom has called by attention to the fact that in a sample of sea water supplemented with organic compounds, and placed in a closed flask with some added clay, a concentration of 151 mg. of hydrogen sulfide per liter was found which must have accumulated from sulfate reduction.

Organic compounds, such as sodium butyrate, acetate, and formate, did not give rise to as good a reduction reaction. An example:

Canal water with 45 mg. of SO_3 per liter was supplemented with 50 mg. of glucose, 100 mg. of calcium phosphate, a drop of iron chloride solution, and 0.5 gm. of sodium carbonate. The reduction proceeded under the influence of an inoculum consisting of the sediment from a previous reduction experiment, as shown in Table IV.

Table IV

Hours after Start of Experiment	Cc. Normal Iodine per liter	Mg. H_2S per liter	Mg. SO_3 per liter	Remarks
12	0.2	3.4	8	Darkening of the liquid
18	0.4	6.8	16	
24	0.5	8.5	20	H_2SO_4 disappeared
36	0.6	11.2	24	Again some H_2SO_4

In this case of 45 mg. of SO_3 per liter, 24 mg. appeared in hydrogen sulfide, and 21 mg. disappeared in various other ways.

If the glucose is increased, one must take care that a butyric acid fermentation does not occur, since granulobacteria are found in canal water in large numbers. For example, if I added 100 mg. or more of glucose per liter, the reduction was greatly inhibited; and in such a medium one finds reduction occurring only after an incubation of a week or a month, if the butyrate and a large amount of carbonic acid disappear.

I do not think it necessary to describe further experiments. The above were selected from a number of similar ones carried out during the years 1893 and 1894.[12] I may add that it seems immat rial which form of sulfate is provided as long as it is not naturally toxic. Sodium sulfate, calcium sulfate, and alum are equally good when appropriately diluted, as are iron sulfate, Mohr's salt, magnesium sulfate, and gypsum. Apparently, we are concerned here only with the SO_4 group as it occurs in the liquid media.[13]

[12]The origins of this investigation were purely practical; they concerned the development of an economical method of obtaining from canal water a completely gypsum-free, potable water for use in steam boilers.

[13]On the basis of the newer theory of solutions, it would be better to express the results of analysis, not as SO_3, but as SO_4.

Isolation and Properties of the Sulfide Ferment

I have spent much time and effort in isolating the sulfide ferment. This is not because the task is especially difficult, once one becomes familiar with these organisms, but rather because I began this investigation on an erroneous assumption, namely, that the bacteria ordinarily found in soil and water would reduce sulfate.[14]

I finally concluded after many negative experiments that this assumption was incorrect, and a special ferment of spiral morphology must be involved, whereupon I fell into another error. I had observed in numerous cultures, especially on solid media, that sulfate reduction was apparently aided by oxygen, I further thought that this must be the case with the sulfide ferment, since other spirilla known to me all required a small, but definite, amount of oxygen for growth.

Using the gelatin plate technique, I was able to isolate three cultures which were varieties of water spirilla, and which I considered to be *Spirillum tenue* Cohn, and which I further believed to be the correct agent; it was not easy to establish with certainty that under no circumstances would these bacterial species reduce sulfate, and I lost much time attempting to confirm the negative characteristic regarding sulfate in these spirilla. For these and other reasons, this investigation has been characterized by many experimental failures.

A step in the correct direction was made with the discovery that the effect of oxygen was not ascribable to its effect on the sulfide ferment, but rather to its effect on related bacteria, which by their growth made conditions satisfactory for the sulfide ferment.

It then became clear that the ferment, although a spirillum, must be an obligate anaerobic organism that develops in a medium containing a limited amount of organic matter; and for this reason the reduction occurs in media freed of oxygen by prior bacterial growth.

In crude cultures the sulfide ferment grew only in small numbers, as contrasted with the ordinary bacterial types; thus a technique was required that would allow better growth of the sulfide bacteria. This

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[14]A beautiful demonstration on the isolation of nitrate-reducing bacteria from crude cultures is the following: One adds to nutrient gelatin 0.1 per cent calcium nitrate and some potato starch, boils it and pours it into a glass plate. After solidification, sterile water and the crude material (a drop of tap water, canal water, etc.) are added. The plate is then inverted, and the water falls off; one then incubates at 20° C. When the colonies have developed, one covers half the plate with dilute hydrochloric acid, in which potassium iodide is dissolved. All colonies which have reduced nitrate thereby have a zone of nitrite around them, wherein they are blue colored on a colorless background. The bacteria covered with hydrochloric acid die. Through a comparison of both halves of the plate one determines the location of other reducing colonies. The numbers of nitrate-reducing organisms growing in canal water are high.

was accomplished with the aid of a small apparatus which I shall call a separation flask; two types are shown in Figures 1 and 2.* The flasks were devised to separate the aerobes from the anaerobes under the usual cultural growth conditions, by development of anaerobiosis in the bulk of the medium; it was assumed that those with demanding

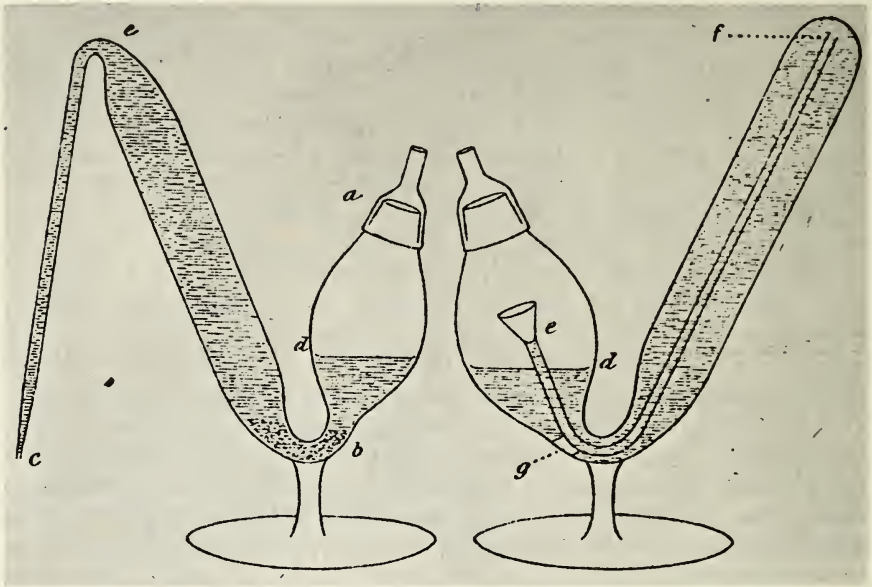

Figure 1 (left). Flasks for the crude separation of anaerobes and aerobes. In the case of sulfate reduction, the iron sulfide is first visible in *b*. From the capillary tip at *c*, an inclined position of the flask allows a rich sulfide ferment-containing culture to be drawn off, whereas in *db*, principally aerobic bacteria occur; *a*—glass cover.

Figure 2 (right). Another form of separation flask. Within a glass-covered, sealed fermentation flask, a glass bead, *g*, is fused to a tube; *e* is a small funnel, and *f* is open. Nutrient medium is in *e* and *d*, and the inner tube will contain anaerobes growing in large numbers; thus, via *e* further culture may be obtained. At the tip of *e*, a drop of paraffin is used as a seal.

*Beijerinck's figures are reproduced by courtesy of Gustav Fischer, Stuttgart. *Editor*.

nutritional requirements would not be motile forms,[15] and provided there was no interference from without, they would not tend to go upward in the vertical column but rather to sink to the bottom of the flask.[16] The flask is a modification of the usual fermentation flask, differing in that it possesses a conducting tube drawn out from the highest point of the gas tube. This can be accomplished in two ways, as is depicted in Figures 1 and 2. In Figure 1 the conducting tube is drawn out to a capillary point, and with the gas tube completely filled, the column of liquid is held up by surface tension and excess pressure so that no back flow occurs; and since the capillary opening is small, entrance of air is prevented. The flasks may be used only where there is no development of gas; otherwise the natural separation, which bacteria of unequal oxygen demand bring about, will be lost.

If in these flasks one cultivates a mixture of bacteria in a given medium, wherein are found aerobes, and obligate and facultative anaerobes, one finds after a time the following division: The aerobes are found in the bulb and remain above a sharply defined line of separation from the bottom of the tube. If one examines the liquid above this line, one finds aerobes without fail, below it they are present only in small numbers. If only aerobes were cultivated in these flasks, the medium in the gas tubes would remain completely clear and translucent, but in the bulb it would be densely turbid. This experiment is quite spectacular when used with such fluorescent bacteria as *Bacterium fluorescens non-liquefaciens* [*Pseudomonas eisenbergii*] cultivated in meat broth.

The facultative anaerobes are distributed either homogeneously throughout the entire flask or else in the bend, where, owing to sedimentation, they are present in enormous numbers.

The obligate anaerobes are generally distributed in the medium, but there are more of them than of aerobes in the gas tube, particularly if the medium is thoroughly mixed.

The results of experiments with these flasks indicate that a strong reduction occurs, and if one allows a drop to flow from the capillary tube, it will be found to contain numerous sulfide spirilla.

The second type of separating flask is shown in Figure 2. In this flask the conducting tube is internal; one end of the tube ends in an open point in the upper end of the tube, while the other ends in a small glass funnel in the bulb of the flask; samples for further investigation

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[15]This is generally valid regardless of motility.

[16]Some bacteria grow in liquid media of specific gravity equal to their own; for example, the lactic ferment is grown in wort solution of 10° Balling. They naturally would distribute themselves homogeneously throughout the entire medium and in the gas tube. The lactic acid bacteria are, however, facultative anaerobes. Aerobic types remain in the same medium of 20° Balling above a sharp line of demarcation from the bottom of the gas tube.

can easily be removed from the funnel, either with a capillary pipette or a platinum needle.

At the start of an experiment, the flasks are filled with sterile, air-free nutrient medium in such a way that the small funnel remains above the level of the medium. It is inoculated in the usual way, and care taken to prevent contamination. The inoculum must be added immediately after cooling the medium; the largest possible inoculum should be introduced into the bulb, and the entrance of air draughts must be prevented. By cooling only the curved portion, not the entire gas tube, the liquid in the upper end of the gas tube is prevented from mixing with oxygen-containing liquid, by flow due to cold water currents. One can successfully inoculate the medium in the bulb at 25°C., whereas that in the gas tube may still be 66°C. These observations hold generally for the flask with the external conducting tube, as shown in Figure 1. If one fears that moisture on the stem of the siphon may lead to contamination of the inner tube by capillary action, one may seal it off with a drop of paraffin. Later the paraffin film may be pierced with a needle or capillary tube, and obligate and facultative anaerobes may be recovered. In the following experiments the outer tube was inoculated with canal mud or sediment from a prior experiment, and there was a noticeable accumulation of the sulfide ferment (with other spirilla) in the inner tube.

Another improvement in the cultural investigation of the sulfide ferment was made on the basis of the observation that in the vicinity of the usual water spirilla the growth of the sulfide ferment was enhanced. Since I had cultivated *Spirillum tenue* Cohn for some time, an application of this organism could be made.

Spirillum tenue grows slowly and with difficulty on the usual media. The medium ought be of a weakly alkaline reaction and contain neutralized salts of organic acids. The presence of peptone is stimulatory. Two of the three spirilla varieties isolated previously did not liquefy gelatin, or did so weakly. In meat broth, beautiful cultures were obtained; some of the spirilla possessed more than twenty turns. The formation of calcium carbonate is a characteristic feature of spirilla, whether grown on solid or in liquid media.

Liquid medium in filled separation flasks yields results as good as those obtained with crude cultures. But I have used still other mixtures. If *Spirillum tenue* is inoculated with the test material, the amount of organic matter in the sulfur solution may be greater than without this bacterium. As observed earlier, the concentration of carbohydrate must be kept low so that fermentation and acid production do not occur.

In all cases the liquid medium was heated before the flasks were filled and again after, to free them of carbonic acid.

The flasks were filled with a medium of the following composition: canal water, to which was added 0.25 per cent calcium malate, 0.25

per cent peptone *siccum,* and 0.1 per cent ferro-ammonium sulfate; it was made alkaline with sodium carbonate and precipitated.

Inoculation of fresh canal water with *Spirillum tenue* resulted in iron sulfide formation after 24 hours at 28°C.

In another experiment the flasks were filled with canal water and 0.25 per cent asparagin, 0.2 per cent magnesium sulfate, 0.2 per cent calcium phosphate, 0.1 per cent ferrous lactate, and 1 per cent sodium carbonate; a weak turbidity resulted. Incubation was as above. If *Spirillum tenue* grew poorly in this mixture, there was soon a rich bacterial proliferation, and after 48 hours at 30°C. there was a strong formation of hydrogen sulfide and ammonium sulfide. Thus 0.5 per cent pure peptone solution, 0.1 per cent ferro-ammonium sulfate, and some sodium carbonate should be included in the medium. I should say that, in general, the higher concentrations of organic matter yielded stronger reductions, provided these were within limits not inhibitory to the reaction. If the organic matter must be decomposed by various other organisms, then a longer period is required before reduction begins. The highest concentrations I investigated were in a mixture of 1 per cent sodium malate, 0.5 per cent asparagin, 0.25 per cent calcium phosphate, 0.25 per cent sodium chloride, 0.25 per cent sodium carbonate, and 0.1 per cent ferro-ammonium sulfate in canal water. After three days at 28°C. iron sulfide was found. I wish to emphasize here that whereas mixed inocula with *Spirillum tenue* were indeed stimulatory, it was not essential for the success of these experiments.

There was found in curve *b* of the flasks containing the liquid medium a flocculant precipitate in which the black spots generated by the sulfide ferment had settled. Later the black coloration increased as a consequence of further iron sulfide formation, and spots were found on the gas tube and walls of the flask. These black flecks built up in vertical streaks, due to the settling of the sulfide ferment, and it is again stressed that motile bacteria under good nutritional conditions are non-motile. As noted above, motile bacteria are usually still and move only when forced by necessity to do so.

Isolation of the sulfide ferment in the flasks may be carried out easily on gelatin or agar plates.

The agar medium was prepared as follows: A very clear, twice-filtered aqueous agar solution was washed for some time with distilled water, thus removing all soluble salts. This was done by repeatedly covering the solidified mass with distilled water which was replaced at intervals. Finally the mass was melted by steam, and an extremely small amount of nutrient solution containing sodium malate, asparagin, and calcium phosphate was added, and the mixture reheated to drive off the air. While it cooled, a drop of clear, neutral ferro-ammonium sulfate was added along with a trace of sodium carbonate, so that no turbidity resulted. Then a small drop of material from the capillary

tip of a separation flask was mixed in well, and without delay the material was placed in a tube or flat glass dish and sealed with a tight cover and cooled in water. The dishes were a few millimeters deep and a decimeter in diameter and were provided with ground glass covers (Figure 3). The agar was poured in such a way that a free space remained around the solidified mass.

In this arrangement a completely oxygen-free nutrient medium is obtained, and it is known that the sulfide ferment, if present, may be cultivated in this manner. Upon inoculation of this medium other bacteria are included and these help in maintaining anaerobic conditions, and they also alter the condition of the medium that after two days or so the sulfide ferment finds conditions for its growth favorable; thence follows sulfate reduction and colony formation. Related spirilla grow well, and colonies of sulfate reducers grow slowly, but in any case reduction proceeds until all of the iron is converted to iron sulfide. If other species besides the spirilla play a role in the reduction, their appearance on this medium was very sporadic and unclear. In experiments in which the reaction was "stormy," reduction ceased after two or three days. In this event, since the colonies of sulfate-reducing bacteria are very small, it is best to begin anew.

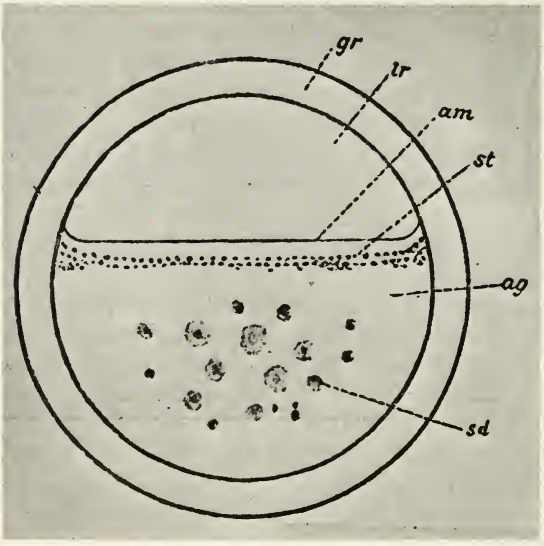

Figure 3. Culture of a mixture of *Spirillum tenue, st,* and *S. desulfuricans, sd,* in agar, ag, between circular glass plates, *gr*. The space between the plates is only partly filled; *lr* is the air space; *am* is the meniscus.

The layer of colonies of *Spirillum tenue* lies below the agar meniscus at st, and they have developed in a zone of diminished oxygen tension. Colonies of *S. desulfuricans* are black due to precipitation of iron sulfide in a zone surrounding them.

In any case, it is advisable to consider only those colonies which grow well, for even so, their numbers hardly exceed a thousand. In these experiments the colonies were apparently large and surrounded by a zone of precipitated iron sulfide; if this was dissolved in acid, the colonies often disappeared entirely. The growth of the culture may be promoted by entirely removing the colony and its agar directly

into a fresh sulfate reduction medium. If this liquid shows change, the growth of the colony is continuing.

Thin agar plates can be conveniently stacked together in wide-necked, stoppered bottles; nutrient solution may be applied peripherally to the agar layers between the glass covers, and the covers will completely keep the air out. Even using these procedures, I have had to work with small bacterial masses, and this demands foresight; for a pinch of inoculum taken from a colony, with a platinum needle, may appear to be a satisfactory inoculum, yet be sterile.

The difficulty resides in the last step of the isolation; for after the culture is isolated, and there is no benefit to be derived from other organisms, an absolutely oxygen-free space must be provided. Our knowledge of the bacteria involved in peptone decomposition has proved helpful, but my findings are not unique; since many of these species are anaerobic, the problems involved in their cultivation are the same as with the sulfide ferment.[17] I have used pure cultures in these investigations only to make definite morphological characterization of the organism involved, and to establish the point that the reduction of sulfate proceeds solely through the mediation of this one organism.

We shall now consider the characteristics of the colonies. They appear similar on gelatin and agar, and no differences were observed; the gelatin was not liquefied.

In the absence of iron salts the characteristic coloration around the extremely small colonies is not formed.[18]

In the presence of iron salts the colonies possess a characteristic appearance (Figure 3) which allows them to be easily identified, but it must be observed that the properties are outstanding for the sulfide ferment only if sulfate is the sole sulfur source; otherwise different bacterial species may produce hydrogen sulfide. However, in such instances the organisms involved would not be spirals, so that further observations with the microscope are recommended. The spiral form of the sulfide ferment is not always sharply evident.

Two types of colonies appear, one with a diffuse zone around it, gradually fading as the distance from the center increases, the other with intense black spots without a zone. It is not clear why there

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[17]I wish to emphasize here that there are two classes of anaerobic bacteria. One class, as represented by the butyl ferment, prefers to absorb a small trace of oxygen, wherefore their morphology is a characteristic one, called an "oxygen type." ("The Butyl Alcohol Fermentation," Amsterdam, 1893, p. 27.) The second class, to which the sulfide ferment belongs, does not possess an "oxygen type" and requires complete absence of oxygen in order to grow.

[18]Zelinsky's *Bacterium hydrosulfureum ponticum* [*Desulfovibrio desulfuricans*] liquefies gelatin and forms a brown pigment.

should be a difference, but I have been able to obtain further reduction only from those colonies surrounded by the zone, not from the other type. Motile cells could be demonstrated in both colony types, so that the difference could not be ascribed to living versus dead organisms. There is probably no constant morphological difference between the bacteria comprising these two types of colonies, and there is no reason for supposing two different bacterial species to be present. Yet I must note that from transfers of the bacterial colonies with the zone, a similar form developed upon subculture.

The zone around the colonies consists, as previously stated, of iron sulfide (Figure 4). The precipitate is symmetrically distributed around the colony and gradually fades away as the distance from the center increases. The precipitate consists of small spheres, mostly of approximately equal size, together with a few irregular clumps. Each small sphere is equal in size to about a thousand micrococci. They are completely soluble in hydrochloric acid. They are not organic concretions (such as result when sodium carbonate and calcium chloride react in the presence of protein or gelatin), but are composed of pure iron sulfide. A few spheres within the colony are distributed among the bacterial cells, but their numbers are limited and the colony proper shows but little coloration.

I have shown in this study that in the separation flasks a number of water-dwelling spirilla usually appeared with the sulfide ferment. Thus it was concluded that the inoculum containing the ferment also contained *Spirillum tenue*. When peptone, or salts of organic acids and ferrous-ammonium sulfate, as indicated, were used, *Spirillum tenue* grew so well that the other bacterial types were more or less completely suppressed. The following sequence of events occurs when an inoculum is made using the glass plate technique, as shown in Figure 3: Where air is present, colonies of *Spirillum tenue*, are formed, and below them, colonies of the sulfide ferment; all other

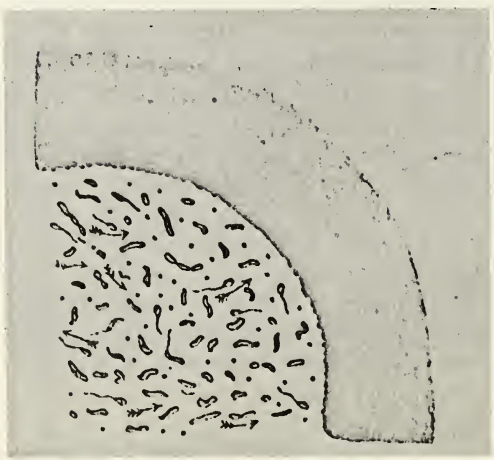

Figure 4. A colony of *Spirillum desulfuricans,* as in Figure 3, but much enlarged. In the agar and within the colony numerous particles of iron sulfide are noted. Some of the spirilla were dead and black. The motion of the cells is indicated by arrows. X 1,300.

bacterial colonies, particularly those of aerobes, are completely suppressed.

I should draw attention to the characteristic growth of *Spirillum tenue* colonies, which, in reference to the proportion of organisms occurring in free air, is of some significance. The colonies appear characteristically in a zone at a certain distance from the agar meniscus, and here they grow to a very definite size (*st,* Fig. 3); above and below this zone no colonies are formed. Obviously the colonies are located in the medium at a level where there is a definite, but limited, oxygen concentration. In another paper I have divided motile bacteria into three types based upon their response to oxygen: the aerobic type, the spirillum type, and the anaerobic type, according to whether the organism in question occurred in the highest, medium, or lowest oxygen concentration. It may be seen that these types of motile bacteria have analogous growth types, and we can infer the one from the other. A further explanation is that with motility, as in growth, an optimal point is reached at the same oxygen tension. The question may be raised as to whether *Spirillum tenue* in a moderate oxygen tension, which is its optimum, decomposes carbohydrate moderately or maximally. The latter possibility is most likely, for with obligate anaerobes the influx of oxygen is prevented by carbon dioxide evolution.

We shall now return to our consideration of the sulfide ferment.

Subsurface agar or gelatin colonies are quite small, and composed of loosely wound spirilla; these ordinarily are about 4μ long and 1μ thick, and rarely are they longer or shorter than this. The number of curves is one-half to one, and only seldom is more than one curve observed. Most cells are very actively motile, provided oxygen does not enter the preparation. If it does, the more actively motile cells migrate to the center, whereas those less active become completely immobile. I have described the same occurrence in the butyl ferment.[19] In the colonies outlined by iron sulfide precipitate, there are, as noted earlier, black micrococcus-like depositions among the organisms. Dying spirilla act as centers of iron sulfide deposition, and they become intensely black and enormously swollen, often completely losing their spiral shape.[20]

In many of the sulfide spirilla, a terminal thread or flagellar bundle is observed only with great difficulty. The forms which occur in the liquid media are mostly smaller than the organisms observed in col-

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[19]*Centralblatt für Bakteriologie,* XIV (1893), 841.

[20]During my investigations I once observed a fine spirillum, which, though living and motile, possessed sulfide granules within itself. R. Koch also appears to have noted this type, and he described it (Koch states, "after M. Perty," but I cannot find this name in *"Smallest Living Forms"*) as *Spirillum leucomelaenum. Mitteilungen des Gesundheitsamts*, I (1881), 48.

onies from solid media, but there is no doubt concerning their spiral shape. I therefore believe that this ferment can be given the name *Spirillum desulfuricans*.

When I use the generic designation *"Spirillum,"* I must emphasize that such a designation is provisional; for it is obvious this organism differs from previously described spirilla. This is especially the case with its behavior toward free oxygen, and in this regard I have come to distinguish three sharply separated types, recognizable by their colonial morphology on solid media as well as the "respiration figure" of the motile types. The aerobic type is exemplified by *Spirillum tyrogenum* (also the cholera organism); the spirillum type is illustrated by *Spirillum tenue;* and the anaerobic type is *Spirillum desulfuricans*.

It is apparent that although these physiological differences are of only remote systematic significance, they might lead to dividing the present artificially constructed genus *Spirillum* into at least three natural species. A time will arrive when we possess a clearer view of the complete bacterial system than we do at present.

When one obtains *Spirillum desulfuricans* in pure culture, one must not be led to assume that further difficulties in studying it will disappear. Quite the reverse, for the difficulties are greater than in dealing with crude cultures. Every precaution must be taken to prevent the entrance of oxygen, and it is very difficult to work with the organisms under conditions where one cannot depend upon the aerobes to bring about anaerobic conditions. Perhaps it would be advisable to use ferrous sulfate instead of ferrous-ammonium sulfate. Yet this is perhaps a last resort.

Although I have worked more than two years with the sulfide ferment, I have been unable, because of such circumstances, to establish with certainty many points of their biology. If I had the necessary time, I believe the final results of a reduction experiment would be obtained after three to four weeks, but in the case of experiments with pure cultures such work would probably fail. We have yet to provide answers to the following questions: Can the sulfide ferment reduce compounds other than sulfate, for example, indigo-carmine, litmus, ferric salts, nitrate? Are the hydrogen sulfide and iron sulfide organisms which are very extensively distributed on clay seashores identical with *Spirillum desulfuricans?* Do the sulfide ferments in our canals and sewers belong to the same group as those found in the soil, or are there several species? Finally, the especially important questions of the occurrence of the ferment in the deep soil layers, and a description of the sites in the soil where sulfate reduction is accomplished, are as yet unsolved problems.

Further Readings

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XVI.

ERWIN FRINK SMITH

(1854 to 1927)

Erwin Frink Smith was born in Gilbert Mills, New York, on January 21st, 1854, and he died on April 6th, 1927, in Washington, D. C. Smith entered the United States Department of Agriculture in Washington in 1886 as an assistant to F. Lamson-Scribner in the Section of Vegetable Pathology. He was a self-educated man, as witnessed by the fact that he obtained a B.S. degree from the University of Michigan (1886) after only one year, the other three years' work being passed by special proficiency examinations. In 1889 he received his doctorate in botany from the same university with a dissertation on "peach yellows." Smith remained at the Department of Agriculture until his death thirty-eight years later.

He began a study of *Fusarium* mold injury in 1894, and in 1899 published the first paper anywhere on this subject. His famous controversy with Alfred Fischer of the University of Berlin, regarding the ability of bacteria to cause plant diseases, dates from 1898. Smith's ultimate victory did much to increase the stature of American plant pathology in Europe.

Smith made great contributions to plant pathology through his numerous studies on bacterial diseases of plants, first on the bacteria per se, and later on the morphological changes they induced in the infected plant. His work on brown rot of tomato (1896), black rot of cabbage (1897), bean blight (1897), and wilt of maize (1898) are models of critical and detailed descriptions of bacterial diseases of plants. The first volume of his monumental *Bacteria in Relation to Plant Diseases* was published in 1905. A second volume appeared in 1911, and the third followed in 1914.

Smith received a certificate from the American Medical Association in 1913 for his paper dealing with cancer in plants. This was a subject that continuously interested him, and he wrote forty papers on various aspects of it. Smith and his predecessor, T. J. Burrill, were two of America's outstanding microbiologists in the field of plant pathology.

American Philosophical Society

Erwin Frink Smith. Courtesy of A. D. Rodgers and L. P. Eisenhart.

Smith's controversy with Dr. Fischer centered about the ability of microorganisms to cause diseases in plants, and is, in some ways, reminiscent of the situation that arose when bacteria were implicated in diseases of man and animals by Pasteur and Koch. As early as 1813, Knight had suggested that mildew or rust of wheat was caused by "a minute species of parasitical fungus which is propagated like other plants by seeds." Fischer insisted that some predisposing injury to the plant was necessary for microbial invasion, and that microorganisms were not primary agents of disease, but, rather, secondary invaders. The following paper, published in 1899, illustrates how Smith disposed of Fischer's arguments. It was published in the *Centralblatt für Bakteriologie*, where it would not fail to come to the attention of the majority of European workers. It appeared in Section 2, Volume V (1899), 271-278.

ARE THERE BACTERIAL DISEASES OF PLANTS?

A Consideration of Some Statements in Dr. Alfred Fischer's *Vorlesungen über Bakterien*

In Dr. Alfred Fischer's *Lectures on Bacteria,* published in Jena in the last part of 1897, occur the following statements which are so contrary to fact that I desire space in the interest of science for some counter statements:

> Apart from nodule bacteria which are singularly related to the legumes previously described, there is not another example known at present in which bacteria are able to enter into the closed living cell of a plant. The uninjured living plant is in contact with the outer world through the stomata, and there is some communication with the outer air, in contrast to the completely closed cells in a system of air-filled interspaces. If bacteria are driven by wind or rain into the stomata openings, they succeed in penetrating the intercellular space, where they are exposed to nothing more than moisture-saturated air, devoid of nutriment; without this, spores do not germinate or bacteria grow. Even if bacteria which attack cellulose (methane bacteria) are in the intercellular space, they cannot grow; and thus their ability to dissolve cell walls does not develop. Only those organisms that are supplied with their own sufficient nutriment when they enter plants can grow in water and attack the cell wall. This is true of spores of parasitic fungi that carry their reserve material in a germ tube which bores through the epidermis of the plant (potato mold, *Phytophythora infestans*) or through the stomata (red mold); and they penetrate the cell wall, grow within the cells, or send their mycelia in special lateral branches as haustoria. Bacteria do not have these

properties, and the plants are completely protected against other forms of invasion.

In an injured plant cell nutriments are available for bacteria, but this source is soon cut off; for under the wound surface an impenetrable cork layer (wound cork) is laid down, and further exudation of sap is halted. The wound does not remain moist, the cells shrink and dry, and the entrance of the bacteria is directly obstructed, as in the uninjured plant. There is no threat of wound infections by bacteria, and dissemination in the plant is unlikely. Regarding injection of pathogens, as in the case of animals and man, the process in plants is easily predicted: no development in the intercellular spaces, or perhaps a proliferation in the injected area that is completely limited and quickly extinguished.

Experiments of this sort always yield these results, and further comment is not required. Yet, again and again we obtain new descriptions of plant diseases, often mere descriptions and often very uncritical work. It is certain that bacteria are found in ailing plants, but they occur only as metatrophs with invading molds and settle on decomposed tissue to further the process, or lead to other abnormalities in the further course of the disease. The primary attack by molds on the plant must occur, like other deleterious effects, such as frost, animal predation, etc., from such wound infections and other incurable hurts, and not merely by illness of the intact plant. From bacillary gummata of grape vines to potato scab, all alleged bacterial diseases of plants have other origins; for bacteria are merely metatrophic contaminants, not "all-conquering parasites."*

It is seldom in a genuinely scientific book that one finds so many unwarranted assumptions and serious misstatements in the space of a single page. The statement in the first sentence is entirely and unqualifiedly erroneous. The statement in the second sentence is not true unless the author includes water pores under stomata. If they are thus included, then the statements in the third sentence are erroneous, since the cavities under the water pores are often filled, not simply with moist air, but with fluid. Moreover, it is not true that "all nutrients are absent" in these substomatic chambers, because the excreted fluid is not pure water, but water holding in solution all the nitrogenous, carbohydrate, and mineral elements necessary for the growth of bacteria, as I have proved in case of the cabbage. Granted this, it will be seen at once that an uninjured plant is not completely protected against bacteria, provided it has water pores which function. Neither is such a plant fully protected if it has floral or extrafloral nectaries. The stigmatic surface is also an unprotected portion.

In case of the black rot of the cabbage, a bacterial disease widely prevalent in the United States, I have shown conclusively that in some fields fully 90 per cent of the total infections take place through the water pores in just the way Dr. Fischer declares to be impossible.

*Translated by the editor.

In pear blight, a bacterial disease widely disseminated in the United States, it has also been shown conclusively by M. B. Waite, one of my colleagues, that a very large proportion of the infections take place naturally through the nectar disk of the blossoms. The parasite finds abundant food for growth in the nectaries of pear and apple flowers and from this coign of vantage readily dissolves its way down into the stem and destroys it.

The statements respecting wound infections are equally erroneous. Dr. Fischer's assumption of what happens when bacteria find their way into wounds, viz., that an impervious cork layer is immediately formed under this wound and the further progress of the bacteria thus prevented, is precisely what does not happen in the case of pear blight, cucurbit wilt, brown rot of potatoes, black rot of cabbages, the yellow disease of hyacinths, the bacteriosis of beans, and all other bacterial diseases of plants which I have found opportunity to study.

I will not deny that these organisms may sometimes be shut out of the host plant by a rapidly formed cork layer, but I have never observed such to be the case, and it is certainly the exception, if it occurs at all. Usually, the parasitic bacterium, when introduced accidentally or purposely into wounds of the plant to which it is peculiar, penetrates into the tissues so rapidly that the plant is soon injured beyond recovery and cannot form any wound cork. Dr. Fischer has never studied one of these genuine plant parasites, and his statements are suppositions of what he thinks ought to happen, rather than a record of what actually does happen in such cases. If the infection of the deeper tissues of a plant takes place so rapidly that no cork layer can form, then the a priori argument against the possibility of the existence of any bacterial wound infection disease also falls to the ground. As a matter of fact, there are many wound infection diseases of plants which are due, principally in some cases and solely in others, to bacteria.

The fact that saprophytes and animal parasites fail to produce any disease when introduced into plants is well known, and no argument as to the nonexistence of bacterial diseases of plants can be drawn therefrom. Indeed, noninfection with such organisms is just what we might expect, the composition of the bodies of living higher animals and of decaying substances being very unlike that of the tissues and juices of living plants. Plants have their own peculiar bacterial parasites, which are often entirely restricted, so far as we yet know, to special families or even genera of plants. There are bacterial plant parasites which appear to be sharply marked off as wound parasites, as much so as anthrax or tetanus. There are others which may be likened to the organisms causing diphtheria, lung tuberculosis, or typhoid fever, since they find their way into the plant body through natural openings. Each of these organisms causes as distinct and specific a series of symptoms in the attacked plants as do any of the animal

pathogenic bacteria in animals. They are also as widely distributed and as destructive as the animal plagues due to bacteria.

Instead of there being no plant diseases attributable to bacteria, if the whole truth were known, there are probably as many plant diseases due to bacteria as there are animal diseases caused by these organisms. I made this same statement more than two years ago and see no reason for retracting it. On the contrary, a wider outlook and a better grasp of the subject lead me to reaffirm it.

I am perfectly willing to admit that several plant diseases formerly ascribed to bacteria are now known to be due to other causes, and also that a considerable number of plant diseases attributed to bacteria have been ascribed so imperfectly that the cause of the disease is still in doubt. These admissions have no bearing on the question at issue, and I say nothing about either of these groups, beyond expressing a belief that some of the latter class will turn out to be genuine bacterial diseases when carefully investigated. I hold simply to clearly established facts and object to having these brushed aside as so much rubbish.

I come now to Dr. Fischer's sarcastic "yet." Inasmuch as I have probably published more on bacterial diseases of plants during the last five years than anyone else, I may be permitted to assume the defensive and to refer particularly to my own investigations. Six diseases of cultivated plants have already been referred to, viz., pear blight, cucurbit wilt, brown rot of potatoes, black rot of cabbages, the yellow disease of hyacinths, and the bacteriosis or water-spot disease of beans. I will add two other plant diseases, viz., the soft rot of hyacinths and the olive tuberculosis. It has been definitely settled that these eight diseases, not to mention several others, are due to bacteria, and the evidence on which this opinion rests is of the same sort and is just as conclusive as the evidence of the bacterial origin of anthrax, glanders, symptomatic anthrax, tuberculosis, diphtheria, hog cholera, typhoid fever, or Asiatic cholera, eight well recognized animal diseases due to bacteria, or at least to well-known organisms commonly classed as bacteria. In passing, it may also be said that it is easier to obtain indisputable results with plants than with the higher animals, since material for infection is always cheap and abundant, so that the experiments may be repeated over and over again at will, which is not at all the case when one is dealing with bacterial diseases peculiar to man.

I select these eight plant diseases because I am familiar with each one, and know from my own experiments in six of the eight that they are due to as many different bacteria. Each of these six organisms I have studied in pure cultures and am familiar with their morphology and cultural characters. The specific bacterial growth is constantly present in each of these six diseases. As a rule, fungi are altogether absent and are never present except as pure saprophytes in secondary

stages of the disease. It is impossible to admit for a moment that fungi pave the way for the bacteria in any one of these six diseases. They are due simply and solely to the entrance into the plant of a specific parasitic bacterium, which multiplies in the tissues because it finds conditions therein suited to its requirements. In five of these diseases I have produced the specific symptoms repeatedly by inoculations from pure cultures; and in the sixth (pear blight), with which I have experimented but little, I have produced the disease whenever I have tried, and have seen it produced by Mr. Waite repeatedly from pure cultures. There are not methane bacteria, but nevertheless all of them dissolve cellulose and form cavities in the host plant. In all of these diseases, in the plants which become diseased as the result of pure-culture inoculations, the specific organism appears in the tissues in enormous numbers, often in pure or almost pure cultures, as sometimes happens in case of the cholera vibrio in the human intestine. This organism may be carried through any number of plants by cultivating it out and reinoculating, just as the germ of anthrax or tuberculosis may be carried through a series of guinea pigs.

In case of the cucurbit wilt, with which I have been experimenting for five years, I must have passed the specific cause of the disease, a peritrichiate bacillus, through nearly or quite three hundred plants, with characteristic symptoms ending in death. Most of these infections were by means of delicate needle punctures (without hypodermic injection), and no protective wound cork ever formed. "Metatrophic contaminants," indeed! Guinea pigs are not more susceptible to anthrax than is *Cucumis sativus* to this organism! I am now preparing in English a full account of this bacillus, but exact and abundant proof of its pathogenic nature was given in 1895 in the German paper referred to below.

In the brown rot of the potato we also have to do with a very active parasite. The introduction of a very minute quantity of this organism into the tiniest wounds on leaves or stems (delicate needle punctures without hypodermic injection) is sufficient to destroy the whole plant, tubers included, in the course of a few weeks. *Phytophythora infestans* is not more destructive. Tetanus is not more certain in its action. Exact and abundant proof of the bacterial nature of this potato disease was furnished by the writer in 1896, and a copy of the paper was sent to Dr. Fischer.

My belief in the pathogenic nature of the organism which I have myself seen associated with the soft rot of the hyacinth and have observed and cultivated out of the interior of the olive knot, rests respectively on the statements of A. Heinz and of L. Savastano. Neither one describes very fully the organism with which he worked, but each was clearly a bacillus and was studied, apparently at least, in pure cultures. Each investigator secured infections with his pure material, conforming apparently to all the requirements necessary to prove the

pathogenic nature of a bacillus, and I see no reason to doubt the published statements.

Dr. W. Migula gives a fairly correct account of bacterial plant diseases in his *System der Bakterien,* which was also published in 1897, and there is no excuse for the unwarranted statements which I have quoted, since the literature of the subject is equally accessible to all. Omitting mention of all papers published in 1897, there remain at least six of these eight bacteria which should have been admitted to Dr. Fischer's book as genuine plant parasites. These are:

1) *Bacillus amylovorus* [*Erwinia amylovora*]. Described by T. J. Burrill in 1880 to 1884 as the cause of pear blight. "Anthrax of Fruit Trees; or the so-called Fire Blight of Pear and Twig Blight of Apple Trees," *Proceedings of the American Association for the Advancement of Science,* XXIX (1880), 583; *American Naturalist,* XV (1881), 527. "Pear and Apple Tree Blight," *Transactions of the Illinois Horticultural Society,* XIV (1880), 157. "A New Species of *Micrococcus* (bacteria)," *American Naturalist,* VII (1883), 319.

Pathogenic nature of the organism confirmed by J. C. Arthur in 1884 to 1887 in numerous papers. "Pear Blight," *3d Annual Report, New York Agricultural Experiment Station* (1884); *Proceedings of the American Association for the Advancement of Science,* XXXIV (1885); "Proof that Bacteria Are the Direct Cause of the Disease Known as Pear Blight," *Botanical Gazette,* X (1885), Nos. 9 and 10; *Gardener's Chronicle,* XXIV, 586; "Pear Blight and Its Cause," *American Naturalist,* XIX, 1177; "Pear Blight," *4th Annual Report, New York Agricultural Experiment Station* (1885), 241; "Pear Blight, Its Cause and Prevention," *Annual Report of the New Jersey State Horticultural Society* (1885); "Pear Blight," *Proceedings of the American Pomological Society* (1885), 44; "Pear Blight," *5th Annual Report, New York Agricultural Experiment Station* (1886), 275; "History and Biology of Pear Blight," *Proceedings of the Philadelphia Academy of Science* (1886), 322-341; "Controlling Pear Blight," *Proceedings of the Western New York Horticultural Society* (1886), 81, etc.

Pathogenic nature reaffirmed as the result of a long series of exact experiments by M. B. Waite, 1891 to 1895, and also in more recent years. "Results from Recent Investigations in Pear Blight," *Proceedings of the American Association for the Advancement of Science,* XL (1891), 315; "The Cause and Prevention of Pear Blight," *Year Book, United States Department of Agriculture* (1895), 295; "Life History and Characteristics of the Pear Blight Bacillus,' *Proceedings of the American Association for the Advancement of Science* (1898) (also printed in *Science,* New Series, VIII).

2) *Bacillus oleae* [*Pseudomonas savastanoi*]. Pathogenic nature asserted by L. Savastano in 1886 and 1887 and proved by the same experimenter in 1889: "Nosologie Végétale.—Les Maladies de

l'Olivier et la Tuberculose en Particulier," *Comptes Rendus,* CIII, 1144; "Tuberculosi, Iperplasie et Tumori dell' Olivo," *Annali della Regia Scuola Superiore di Agricoltura,* Portici, V (1887), Fasc. 4; "Il Bacillo della Tubercolosi dell' Olivo," *Rendi conti della Reale Accademia dei Lincei,* Rome (1889), 92.

3) *Bacillus hyacinthi-septicus* [*Erwinia carotovora*]. Pathogenic nature established by A. Heinz in 1889: "Zur Kenntnis der Rotzkrankheiten der Pflanzen," *Centralblatt für Bakteriologie und Parasitenkunde,* V, 535.

4) *Bacillus tracheiphilus* [*Erwinia tracheiphila*]. Pathogenic nature established by Erwin F. Smith in 1895: "*Bacillus tracheiphilus* sp. nov., die Ursache des Verwelkens verschiedener Cucurbitaceen," *Centralblatt für Bakteriologie und Parasitenkunde,* Section 2, I, 364.

5) *Bacillus solanacearum* [*Pseudomonas solanacearum*]. Pathogenic nature established by Erwin F. Smith in 1896: "A Bacterial Disease of the Tomato, Egg Plant and Irish Potato," *Bulletin No. 12, Division of Vegetable Physiology and Pathology, United States Department of Agriculture* (1896). See also reviews in *Botanische Zeitung, Zeitschrift für Pflanzenkrankheiten,* etc.

6) *Pseudomonas campestris* [*Xanthomonas campestris*]. Pathogenic nature established by L. H. Pammel in 1895: "Bacteriosis of Rutabaga (*Bacillus campestris* n. sp.)," *Bulletin No. 27, Iowa Agricultural Experiment Station* (1895).

Professor Burrill and Dr. Arthur both observed the blighting blossoms caused by *Bacillus amylovorus,* and the latter suggested that the bacteria entered through the nectaries. Subsequently, Mr. Waite proved this by exact experiments. My own statements, respecting the entrance of *Pseudomonas campestris* through the water pores of cabbage leaves, with full proof of the same, may be found on page 408 and the following pages of *Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten,* Section 2, III, where this organism is described and full proof furnished of its parasitic nature. Additional evidence is given in *Farmer's Bulletin No. 68,* "The Black Rot of the Cabbage," published by the United States Department of Agriculture, January 8th, 1898. Since the publication of these two papers, my statements respecting the pathogenic nature of this organism and its ability to enter the plant through the water pores have been fully confirmed by Dr. H. L. Russell in a paper published by the Wisconsin Experiment Station. On this disease, see also *Pseudomonas campestris* (Pammel) Smith: "Die Ursachen der Braun-oder Schwartz-Trocken-Fäule des Kohls," *Zeitschrift für Pflanzenkrankheiten,* VIII, No. 3.

Statements respecting the pathogenic nature of *Pseudomonas*

(*Bacterium*) *hyacinthi,* which always seemed to me to be reasonably conclusive, were made by J. H. Wakker in 1883 to 1889, in the following publications:. "Vorläufige Mitteilungen über Hyacinthenkrankheiten," *Botanisches Centralblatt,* XIV, 315; "Het Geel of Vieuwziek der Hyacinthen Veroorzaakt door *Bacterium hyacinthi* Wakker," *Onderzoek der Ziekten van Hyacinthen,* etc. (1883); *Onderzoek,* etc. (1884); *Onderzoek,* etc. (1885); "La Maladie du Jaune, ou Maladie Nouvelle des Jacinthes, Causé par le *Bacterium hyacinthi,*" *Archives Néerlandaises des Sciences Exactes et Naturelles,* XXIII (1889), 1-25. During the last two years I have fully confirmed Dr. Wakker's statements respecting the parasitic nature of this organism, and will publish a full account of my experiments in the near future. A brief statement has already been published in *Proceedings of the American Association for the Advancement of Science* (1897), where may also be found an account of the bacterium which I have proved to be pathogenic to species of *Phaseolus.*

Complaint has sometimes been made that Englishmen and Americans publish without knowing what has been done in Germany. Insofar as this has occurred, it is always a matter for regret, since Germany is the acknowledged leader of the world in botanical and bacteriological investigation. Here, however, is a counter case of a Professor in one of the most renowned of the German Universities who does not think it necessary to read English, and, still worse, who writes dogmatically on a subject without exact knowledge, and who is not familiar even with the readily accessible literature in his own language. Considering this performance, one feels like quoting a well-known line from Goethe: "Some strike the wall with a hammer and believe that they hit the nail on the head every time."

Further Readings

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Rodgers, A. D. *Erwin Frink Smith: A Story of North American Plant Pathology.* Philadelphia: American Philosophical Society, 1952.

Smith, E. F. *Bacteria in Relation to Plant Diseases.* Washington, D. C.: Carnegie Institution of Washington, 1905, Vol. I.

Smith, E. F. "Entgegnung auf Alfred Fischer's 'Antwort' in Betreff der Existenz von durch Bakterien Verursachten Pflanzenkrankheiten." *Centralblatt für Bacteriologie,* Section 2, VII (1901), 88-100; 128-139; 190-199.

True, R. H. "Erwin F. Smith, 1854-1927." *Phytopathology,* XVII (1927), 675-688.

XVII.

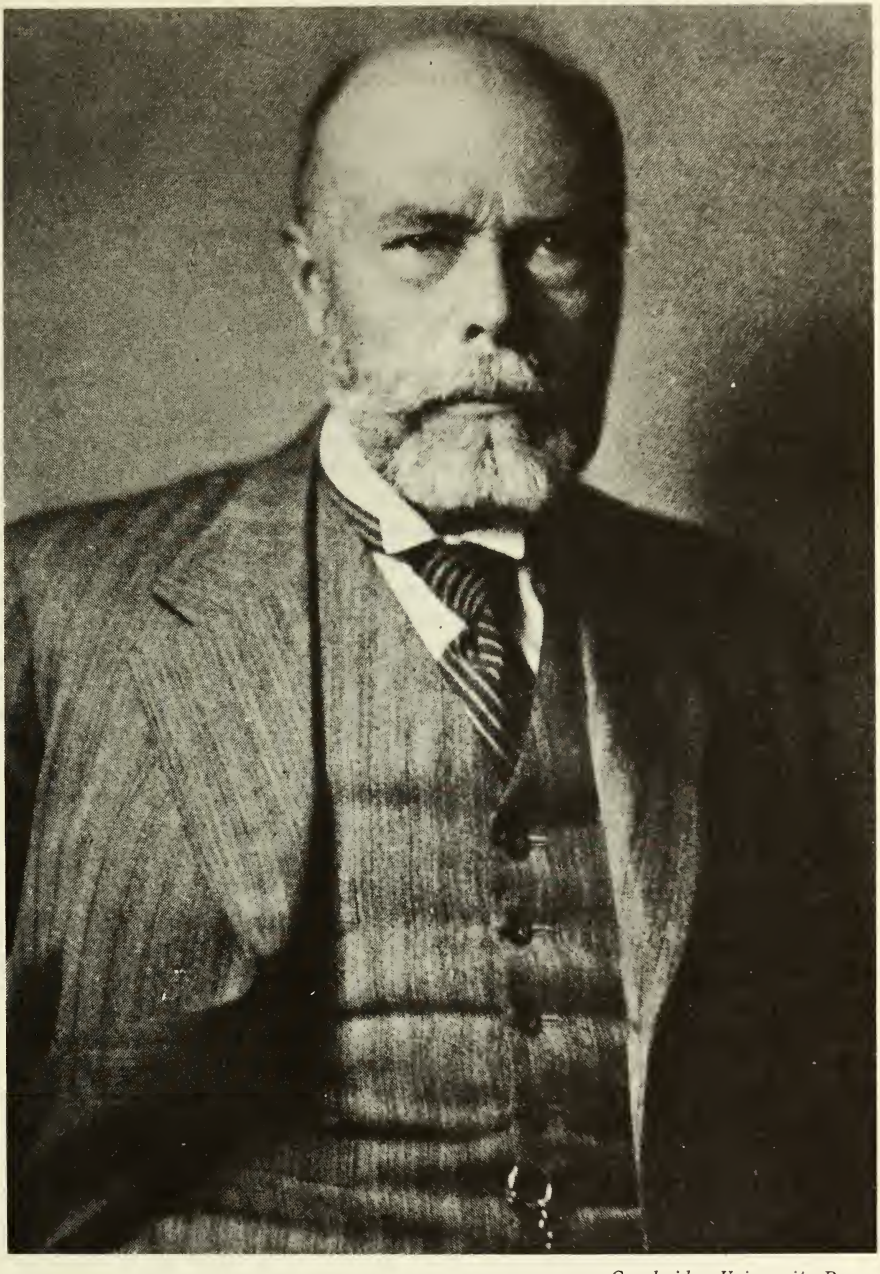
SIGURD ORLA-JENSEN

(1870 to 1949)

Sigurd Orla-Jensen's early training in Copenhagen was directed to chemical engineering, but soon he became interested in microbiology and fermentations. He worked with E. von Freudenreich at Bern, and succeeded him upon his death as director of the Swiss dairy experimental station. In 1906 he was appointed to the Royal Technical College, and in 1908, at the age of thirty-eight, he was made a full professor. He held the latter position for forty years. In 1908 he proposed the "natural" classification of bacteria, that is, classification based upon physiological instead of just morphological characteristics. In 1912 he introduced, with C. Bartel, the methylene blue reduction test for the bacteriological grading of milk, and he was prominent in efforts to have all market milk pasteurized. Orla-Jensen did a great deal of fundamental work on cheese ripening, lactic acid bacteria, and bacterial classification. His classical *The Lactic Acid Bacteria* was published in 1919 and is a model of careful taxonomic study.

He was born on November 26th, 1870, and died on June 24th, 1949, in Copenhagen, Denmark.

Orla-Jensen maintained that the physiological characteristics of bacteria were of primary importance in classification. These characteristics, together with the arrangement of flagella (but not the presence or absence of flagella), were used by him to divide the bacteria into the *Cephalotrichinae* and *Peritrichinae*. He considered those organisms which derived their energy from inorganic sources to be more primitive than any other group. Not only did he propose a system attempting to show phylogenetic relationships but also he introduced an entirely new nomenclature. The following paper was written soon after Orla-Jensen was appointed professor at the Royal Technical College in Copenhagen, and it appeared in the *Centralblatt fur Bakteriologie*, Section 2, XXII (1909), 305-346, entitled "Die Hauptlinien des Natürlichen Bakterien-Systems."



Cambridge University Press

Sigurd Orla-Jensen

THE MAIN LINES OF NATURAL BACTERIAL SYSTEMS

Minerals are classified primarily on the basis of their chemical constitution and secondarily on their crystal structure; and living things should be classified in the same manner, on the basis of their internal development rather than their external appearance.[1] This should hold especially for bacterial classification; many bacterial species, because of their small size, lie just within the bounds of visibility, despite great progress in techniques of microscopy.

Nevertheless, systematists still consider only the morphology of bacteria. Essential important points to them are whether a short rod is longer than it is wide, or whether it is more or less curved. Even with yeasts such characteristics are considered important in formulating genera, but not families, and it is difficult to see how one would propose to deal differently with bacteria. It is normally accepted that in every bacterial family, coccus and spiral forms develop next to short rod species, and there are, in fact, sharply defined families, as for example, the red sulfur bacteria, in which there occur not only these three chief forms but transitions between them.

Bacteria divide in one or more directions, and great systematic significance has been attached to this point. One need not place too great emphasis merely on cell form, for the direction of division must also be considered. As a rule the direction of division is at a right angle to the longitudinal axis of the cell, and if the cell possesses no definite longitudinal axis, division may take place in different directions. *Micrococcus* arose from *Streptococcus,* and from the *Micrococcus* developed *Sarcina.* Thus we find different modes of division in groups of the same form. In *Azotobacter* and *Crenothrix* one observes division first in one direction and later in three directions; and in the purple bacterium *Lamprocystis,* division occurs first in three and later in two directions.

At least as much systematic importance must be assigned to the circumstance of spore formation as to morphological properties. For example, the principal representatives of sporogenous bacteria, the hay bacillus and the butyric acid bacteria, form biologically related characteristic groups.

Bacterial motility has no great systematic significance, for since the investigations of D. Ellis we must suppose that bacterial species can easily pass over to motile forms. Apparently all motile forms of bacteria were originally marine types, and if at present certain groups

[1] The first governs the second, but not vice versa.

(for example, lactic acid bacteria and many pathogenic bacteria[2]) are constantly found to be non-motile, it may merely mean that they have developed for so many generations in a favorable medium that motility is superfluous.

Systematic significance does attach to the mode in which flagella are inserted, and, as will be seen later, I have partly based my nomenclature on this important point. Rod-shaped organisms with polar flagella will be described as *Monas*, whereas those with peritrichous flagella are designated as *Bacterium*.[3] Spore-forming rods are called *Bacillus*. *Vibrio, Spirillum, Streptococcus, Micrococcus,* and *Sarcina* are used in the usual sense. I coined my generic designations from the preceding morphological terms by the addition of prefixes of one or more syllables which reflect the biological characteristics of the bacteria.

Cultural observations provide important indices for judging the inner characteristics of bacteria. Nutritional requirements and the greater or lesser need for oxygen indicate something of the enzyme content of the various bacterial species. On the basis of cultural characteristics, bacteria may be divided into the following groups:

1) Bacteria which, like green plants, require neither organic carbon nor nitrogen sources. These so-called autotrophic bacteria can synthesize their carbohydrates and proteins from carbon dioxide and inorganic salts.

2) Bacteria which require organic carbon sources but do not require organic nitrogen. These forms are able to synthesize protein from carbohydrates (or organic acids) and ammonia, nitrogen, or nitrates.

3) Bacteria which, like animals, require organic sources of both nitrogen and carbon. They are unable to synthesize carbohydrates or proteins from inorganic sources. If we draw conclusions regarding the synthetic capabilities of bacteria from their nutritional requirements, we must presume that their cells are constructed of carbohydrates and proteins. The cells also contain fat, but there is less interest in this, since, as in plants, it is normally formed as an intermediate product.[4] Numerous analyses of bacterial cells reveal that 52 to 80 per cent of their dry weight is protein, and that mucin and chitin are present, but not cellulose. Bacteria are related to lower animals in their chemical composition; and in their morphological

[2] We must recall in this connection that many parasitic animals are more or less non-motile.

[3] The same descriptions apply to the non-motile forms, as well as to the related motile form. Thus I designate, for example, *Nitrobacter*, as *Nitromonas*, and there can be no doubt of a near relationship with *Nitrosomonas*.

[4] In this work, the word plant refers to chlorophyll bearing organisms; and we ignore the non-chlorophyll saprophytic and parasitic plants, as they are further evolved from the true plants.

relations, the thread-forming bacteria show great similarity to the blue-green algae, so that there is no doubt of the intermediate position of bacteria between animals and plants, and therefore many of their previously mentioned biological characteristics are similar to those of plants or animals. One may further conclude that bacteria are more simply constructed than animals or plants, inasmuch as the latter two groups must have their origin from the bacteria.

Before we take up our main subject, we must discuss the question of whether animals are of more primitive origin than plants. Animals appear to be of more primitive origin from a morphological point of view; for simple flagellates are quite similar to common water bacteria possessing polar flagella (a similarity often expressed by describing these bacteria as *Monas*), whereas plants find a natural common relationship with the more highly developed thread-forming bacteria. The contrary opinion will be arrived at if one follows the superficial biological argument: "Obviously the first organisms on earth must have developed in the absence of organic matter; since animals cannot do this, plants must be more primitive than animals. All animals live directly or indirectly through plant-eating animals or plants; therefore no flora, no fauna." If we review the previous considerations that autotrophic bacteria were the first living forms, this argument loses much of its force. Bacteria are food sources for the protozoa. The protozoa serve in like manner the rotifers, and these the crustaceans and insect larvae; crustaceans and insects further serve the fishes, amphibia, reptiles, and birds; and finally the latter are the food of the mammals.

From a purely nutritional standpoint, there is no objection to considering that all eight series of the animal kingdom developed prior to plants. Still, so one-sided a development would have been quite unlikely, for without the activity of plants, an oxygen deficiency would have followed, especially since the atmosphere in the earlier periods of the earth's history contained large concentrations of carbon dioxide. It is unlikely therefore that a kingdom of animals existed before the development of plants. Geological investigations reveal that, at most, animals are as old as plants, but one finds such highly developed forms as *Eurypterus* and *Pterygotus* in Algonkian deposits, where there are no known traces of plant materials. In all likelihood, then, plants did not develop quite so early as animals particularly since in the early history of the existence of living things it was still dark on the earth. At temperatures of between 50° to 70° C. the earth must have been engulfed in a fog so dense that only a few light rays penetrated to the earth's surface. Until light of a certain intensity appeared, it is difficult to assume that there were organisms provided with chlorophyll or other pigments enabling them to use radiant energy; for as soon as this happened, the conditions for further development of the plant kingdom would have been laid. The conclusion to

be arrived at from this discussion is that lower animals apparently existed before lower plants, but that a luxuriant plant development (as during the era of coal formation) was necessary for the furtherance of the animal kingdom.

These propositions yield valuable insights on bacterial physiology. One might conclude that typically putrefactive bacteria which now require animal residues are not as primitive as those bacteria preferably living on plant residues, which as a rule are the usual carbohydrate fermenters. A natural development does not allow such conclusions. After the bacteria of the first group (see the biological divisions), there followed those of the second, and finally the third. We must not forget that protein may serve as a carbon as well as a nitrogen source. Many species of the third group, like meat-eating animals, may grow on proteins (or peptones) alone, and not only obtain thereby the necessary energy, but also synthesize their carbohydrates (as in cell wall synthesis) and fats. Proteolytic tendencies are correlated with increased ability to produce proteolytic enzymes; thus gelatin-liquefying bacteria are as a rule derived from non-liquefying ancestors.

The following points of view derive from such a line of reasoning. Since only the relatively late-arriving mammals synthesize lactose, the lactose-fermenting organisms must be younger than species that cannot attack this carbohydrate. We must assume also that the typical urobacteria are of recent origin. Further, harmless parasites (for example those in the intestine, respiratory tract, and genitals) arose from saprophytes, and pathogenic forms from the harmless parasites. When a pathogenic bacterium becomes avirulent, it is simply an example of atavism. Most of the specific infectious diseases of man are caused by organisms of most recent origin. They are the most distant branch of the great generic tree of bacteria, and thus they are of little interest to us in the present context. We might assume later origins for pathogenic bacteria which require blood serum, or which do not grow well on ordinary media; but since they can live saprophytically, they may, like other pathogenic bacteria, have older origins.

The above considerations emphasized the importance of anabolic processes in bacteria; now we shall consider their catabolic activities. These considerations will lead us into the true fermentations, in which there are three phases: the assimilation of energy sources, cleavage and oxidation thereof, and the formation of fermentation end products. Assimilation and formation of end products were originally considered as simple diffusion processes, and cleavage and oxidation were supposed to be the real manifestations of life; but we now know the facts to be quite different. E. Overton has shown that whereas the external membrane of the protoplasm of plants and animals is permeable to water, it is not permeable to materials that are soluble in oil or fat. The living cell is therefore impermeable to colloidal substances as

well as to sugars and most protein degradation products and salts.[5] The fact that such materials do pass through permeable membranes is a manifestation of life as yet to be explained.

Cleavage and oxidation are processes not necessarily dependent on the life of the cell, but rather on chemical substances, the enzymes, which may continue to be active after the death of the cell. In reactions requiring energy for synthetic purposes, the process must occur within the protoplasm itself. Spurious fermentations[6] are simply hydrolyses, which at best allow a substance to be more easily assimilated into the cell. This reaction is essentially an extracellular operation. This is the case for most bacteria and higher animals. In some lower animals that do not possess a digestive tract, but yet are about the same size as, for example, bacteria, an excretion of proteolytic enzymes would be uneconomical, and often would fail to achieve its purpose completely; thus they take in nutrients *in toto*. They also lack definite cell walls.

If one were inclined to a narrow morphological point of view, and attributed higher development to organisms with definite cell walls rather than to those not so equipped, then it must be assumed that as far as taking in nutriments, lack of a definite cell wall is a necessary condition for survival of the latter. From a biological point of view, lack of a cell wall (as in the amoeba) is required for carrying on an independent existence, for even when the cell plays a role in the life processes of a higher organism (as, for example, the leucocytes), these organisms are, of course, more primitive than those with a definite cell wall. As previously mentioned, bacteria which live off complex compounds produce exoenzymes, whereas autotrophic bacteria need only endoenzymes. In some cases certain acids take the place of exoenzymes, serving to bring inorganic nutrients into solution, for example nitric acid with the nitrifying bacteria, sulfuric acid with the sulfur bacteria, and carbonic acid in plants.

In the next 15 pages, Orla-Jensen discusses the important oxidations and fermentations carried out by bacteria in terms of chemical reactions and energy liberated. The reactions considered are: (1) oxidation of hydrogen; (2) oxidation of carbon compounds (as methane, carbon monoxide, or organic carbon compounds); (3) oxidation of nitrogen compounds; (4) oxidation of sulfur compounds; (5) denitrification; (6) desulfuration; (7) lactic acid fermentation; (8) alcoholic fermentation; (9) succinic acid fermentation; (10) propionic acid fermentation; (11) butyric fermentation; (12) synthesis of gums and mucins

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[5] We shall mention the exceptions to this later.

[6] Regarding true and false fermentations, see my recent report, "Studies on the Enzymes in Cheese." *Centralblatt für Bakteriologie,* Section 2, VI (1900), 838.

(slimy fermentation); (13) amino acid fermentations; and (14) ammonia (urea) fermentation. A summary of the energy liberated in these reactions also is given. Editor.

It is evident that the first bacteria on earth must have been autotrophic, but it is difficult to determine which autotrophic type is most primitive, since energy sources such as hydrogen, methane, hydrogen sulfide, carbon monoxide, and ammonia are products of volcanic action, and therefore all were present in quantity early in the history of the earth. We possess one clue, however, if we make the assumption that the easier a synthesis proceeds the earlier it must have begun.[7] Since H. Kaserer[8] has shown that pure cultures of autotrophic bacteria grow better when a trace of formaldehyde is included in an inorganic liquid medium, one may assume that this compound is an intermediate in the carbon metabolism of autotrophic bacteria as well as plants. In the following equations are given the calories produced or required in the formation of a molecule of formaldehyde, CH_2O, by important autotrophic bacteria when they use the least possible amount of the energy source.[9]

Methanomonas:	$CH_4 + O_2 = CH_2O + H_2O$ 21 40 69	+ 88 calories
Nitrosomonas:	$(NH_4)_2CO_3 + 2O_2 = CH_2O + 2HNO_2 + 2H_2O$ 222 40 2x30 2x69	+ 16 "
Carboxydomonas:	$2CO + H_2O = CH_2O + CO_2$ 2x29 69 40 103	+ 16 "
Hydrogenomonas:	$CO_2 + 2H_2 = CH_2O + H_2O$ 103 40 69	+ 6 "
Sulfomonas thioparus:	$CO_2 + 2H_2S = CH_2O + H_2O + S_2$ 103 2x7 40 69	- 8 "
Nitromonas:	$CO_2 + 2KNO_2 + H_2O = CH_2O + 2KNO_3$ 103 2x89 69 2x111	- 88 "
Green plants:	$CO_2 + H_2O = CH_2O + O_2$ 103 69 40	-132 "

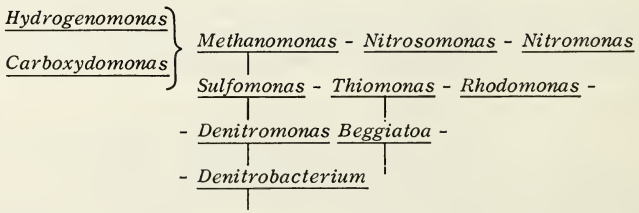
[7]Although I suggest this hypothesis, I hasten to add that regardless of its relevancy it plays no role in the system. The reason that I do not consider the various autotrophic bacteria as isolated forms is that, in my opinion, a preliminary classification based upon a natural principle is better than no order whatsoever.

[8]*Zeitschrift für das Landwirtschaftlache Versuchswesen in Osterreich,* X (1907), 37. I do not accept Kaserer's interesting suggestion that autotrophic bacteria use their own synthesis products as energy sources.

[9]If one adds 10 calories to the right side of these equations, one arrives at the energy required to produce one-sixth of a molecule of fructose (heat of formation, 300 calories).

It is obvious that the carbon assimilation reactions of the first four organisms are exothermic, whereas those of the last three, including green plants, are endothermic. The carbon assimilation reactions of all autotrophic bacteria are accompanied by oxidation or reduction processes which proceed optimally in the dark; plants, on the other hand, evolve elemental oxygen in such assimilations, and since this constitutes a caloric loss, the reactions take place with the aid of the radiant energy of the sun. The above calculations indicate that hydrocarbons are the most suitable compounds in carbon assimilation reactions. *Methanomonas* is doubtless the autotrophic bacterium that can most readily synthesize its cell constituents from such compounds, and it should be recognized as the precursor of all other bacteria. All other organic constituents can be synthesized from hydrocarbons and other reactions.

Thus one may seek the beginnings of organic life in *Methanomonas*, *Carboxydomonas*, and *Nitrosomonas*, for they are approximately equal in the amount of energy liberated during carbon assimilation; we may assume that from *Methanomonas* there developed bacteria that oxidize carbon compounds, on the one side, and, on the other, those that oxidize inorganic nitrogen compounds. Later, bacteria developed that were capable of oxidizing hydrogen and hydrogen sulfide. Since Kaserer's observation that *Carboxydomonas* may oxidize hydrogen if grown in the presence of other bacteria, one must consider that it developed as the *Hydrogenomonas*. After it is decided how *Nitromonas* assimilates carbon, those bacteria that follow *Nitrosomonas* may be placed. This reasoning is probably sound, for it is obvious that until *Nitrosomonas* had oxidized the greater part of ammonia on the earth's surface to nitrous acid, *Nitromonas* could not develop. Continuous rainfall (due to condensation of the large amounts of water vapor in the atmosphere) prevented the ammonia concentration from becoming inhibitory to *Nitrosomonas*. After the formation of nitrites and nitrates, the environmental conditions were satisfactory for the development of denitrifying bacteria, as well as for the true sulfur bacteria, such as the *Sulfomonas* group. In order to make these thoughts clearer, they may be set down as follows:



In the presentation of this scheme we have not as yet examined the morphological details of the bacteria, because these, as I have stated initially, are of secondary importance. A classification based on more or less fortuitous similarities obviously will not reflect natural relationships with any degree of validity. As might be expected, natural relationships show that the most primitive bacterial representative possessed the simplest possible morphology; that is, insofar as we know, they were monotrichous short rods. They were asporogenous and non-branching. Spore-formation and branching come with later developments.

Lophotrichous bacteria probably arose from monotrichous forms, and although the latter are more numerous, there is no sharp separation between the two forms; they belong to the same family, possibly the same genus.[10] The peritrichous bacteria arose from these groups, but, as will be seen later, their pronounced ability to attack proteins and carbohydrates makes them a rather complete group. The entire bacterial world may be divided into two orders, *Cephalotrichinae*[11] and *Peritrichinae*. We shall review the characteristics of each of these orders in turn.

Among bacteria capable of oxidizing inorganic nitrogen, none can be placed before those previously mentioned.[12] They occupy a special place since all the other organisms reduce nitrates. The reduction occurs because energy is liberated, as is the case with the abundantly distributed denitrifying bacteria when the nitrogen for protein synthesis is in an appropriate form, as, for example, amino acids.[13]

Those bacteria which oxidize carbon compounds (without liberating large quantities of further oxidizable products) constitute another series. A transition leads from *Carboxydomonas* to acetic acid bacteria, and thence to those able to oxidize nitrogen[14]—*Azotobacter* and *Rhizobium*. Since these two are polar flagellates, we shall designate

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[10]Doubtless the monotrichous *Bacillus fluorescens non-liquefaciens* [*Pseudomonas eisenbergii*] and the lophotrichous *Bacterium syncyaneum* [*Pseudomonas syncyanea*] belong in the same genus.

[11]The Greek word Kephalos signifies not only head, but more generally the extreme end of an object. *Cephalotrichinae* means a terminally flagellated bacterium.

[12]Neither acetic acid bacteria nor the aerobic nitrogen group are able to oxidize ammonia to nitrite or nitrate.

[13]In order to synthesize the amino group $\overset{'}{C}HNH_2$ from $\overset{'}{C}H_2$ or $\overset{'}{C}HOH$, -NH and NH_3, respectively, are required.

[14]Since the work of F. Löhnis (see *Centralblatt für Bakteriologie*, Section 2, XIX [1907], 87), it is evident that the ability to fix nitrogen is so widely distributed that one must almost presume that any bacterium capable of using an inorganic nitrogen source also may fix atmospheric nitrogen. This property has systematic significance if the bacteria have the other properties in common, as referred to previously.

them as *Azotomonas* and *Rhizomonas*. My investigations have shown that the former, like the acetic acid bacteria, can utilize ethanol as a side carbon source, whereas *Rhizomonas Beijerinckii* as well as *Rhizomonas radicicola* [*Rhizobium leguminosarum*] require higher alcohols or carbohydrates. Both organisms reduce nitrates to nitrites and partly to ammonia.[15] Nitrate metabolized in this way may give rise to ammonium nitrite, which, as is well known, can easily separate in nitrogen and water. This reaction proceeds only if protein can be synthesized, for a nitrogen loss does not occur, as demonstrated by the thorough experiments of J. Stoklasa. With the exception of *Rhizomonas,* we shall group all the previously mentioned aerobic organisms in the family *Oxidobacteriaceae.*

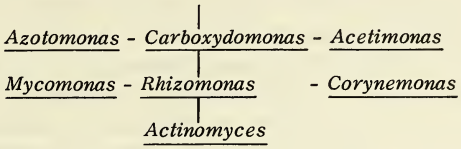
Azotomonas and *Rhizomonas* form pleomorphic cells much like those of the acetic acid bacteria. Strongly pleomorphic nodule bacteria are of interest because they are classified next to *Actinomyces* (*Streptothrix*), which also are root and soil forms. The development proceeds from the root yeasts of the conifers, alders, and other plants, to the *Eumycetes,* and there is some question as to whether the *Actinomyces* ought to be included here instead of with the bacteria, since usually the latter do not produce pleomorphic forms. The relationship would be based not only on branching but on the formation of oidiâ. If this process is carried out in aerial mycelia, a regular arrangement of true conidia result; if it proceeds in the vegetative mycelium, yeast-like cells are formed. In the so-called *Fungi Imperfecti* the most primitive true *Mycomycetes* are found; in this latter group, even as in bacteria, ability to produce spores indicates a later evolution. If we assume that asporogenous yeast cells gave rise to sporogenous forms, the development must have been from *Mycoderma* through *Torula* and thence to *Saccharomyces,* that is from aerobiosis (oxidation), which is usual in *Eumycetes,* to anaerobiosis (fermentation); and (with the benefit of hindsight) from these forms possessing few enzymes to those richly supplied. Since this matter goes beyond the scope of this paper, we shall not pursue it.

K. B. Lehmann and R. O. Neumann conclude the family *Actinomycetaceae* with the genera *Corynebacterium* and *Mycobacterium,* which I shall rename *Corynemonas* and *Mycomonas.* Since they are aerobic, they fit well into the system, and since they do not develop branching of any consequence, one may consider them as among the oldest two genera of *Mycomycetes,* that is among *Rhizomonas.* The relationship to *Mycomonas* seems natural, since as L. Hiltner demonstrated, the

[15]The root nodule bacteria grow only with difficulty in gelatin (those isolated from lupines). *R. Beijerinckii* [*Rhizobium leguminosarum*] gives a weak nitrate reduction, whereas *R. radicicola* [*Rhizobium leguminosarum*] isolated from red clover does not reduce nitrate in liquid culture, and only after long incubation on a solid medium.

relationship between nodule bacteria and legumes is a struggle or invasion, and the similarity of the nodules of legumes to tubercles in animals may be greater than we are willing to believe in these modern times.

This section of the classification system appears as follows:



The *Azotomonas* group, which preferably lives in association with algae, brings us to the probable origin of aquatic plants, and the nodule bacteria and the *Actinomycetes* bring us to the origin of land plants. Proceeding further to the *Mycomycetes* and especially to yeasts, one finds that they develop luxuriantly in association with sweet and succulent fruits.

We shall now turn back to the time when the earth was still dark, and consider the colorless sulfur bacteria. E. Warming, and later G. Hinze, have described elliptical and spherical forms, and M. Jegunow discovered a short rod, and W. Omelianski a spiral form of these bacteria. The *Sulfomonas* group, which consists of short rods, and the thiobacteria, which are rod-like (ovals) *Thiomonas*, must be the most primitive of these bacteria. There arose from these *Thiospirillum* on one side and *Thiococcus* on the other. The red sulfur bacteria (for example *Bacterium Chromatium vinosa* [*Chromatium vinosum*]) form a natural link between *Thiomonas* and *Rhodomonas*, and thence we go into the great family of sulfur bacteria which includes representatives of all morphological types. In this family one finds the usual *Monas* form (as in *Rhabdomonas* and *Rhododictyon*) with cuspidate ends, and also cocci that divide in one, two or three planes, and the above-mentioned intermediate form *Lamprocystis*. I have reserved the prefix *Thio* for the colorless sulfur bacteria, and it is suggested that *Rhodo* be employed for all red sulfur bacteria. The coccus and spiral forms may be placed on each side of the *Monas* type, thus dividing motile from non-motile forms, and the generic chart of the rhodobacteria below (based on Winogradsky) will offer no difficulties.

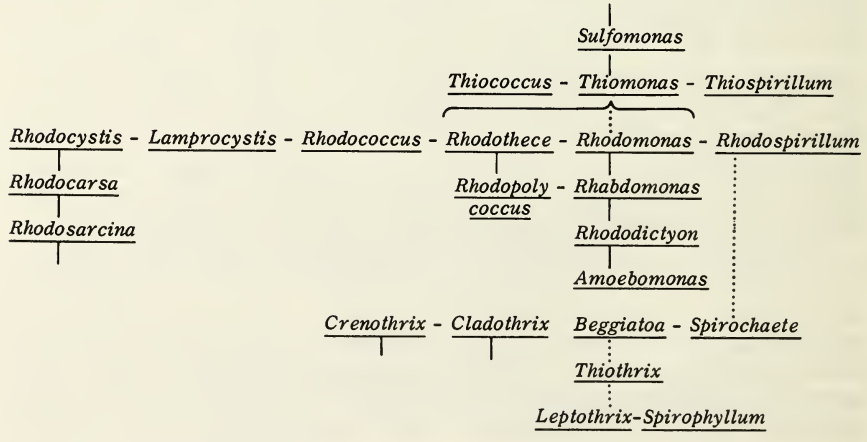
We possess no knowledge of the transitional forms which undoubtedly exist between the swimming thiobacteria and the creeping *Beggiatoa* group. The spiral forms of *Beggiatoa* may be included with the spirochetes. Those spirochetes which live in association with sulfur bacteria in swamp water are the oldest representatives of their group, whereas *Spirochaete pallida* [*Treponema pallidum*] is the youngest and most refined representative. Since the separate thread forms of

Thiothrix also show creeping motility, we may assume that this group arises from *Beggiatoa.*

Although *Leptothrix (Chlamydothrix)* shows some similarity to *Thiothrix,* it is a later development than the parallel forms of the motile, sulfur-free bacteria. The recent investigations of Ellis have shown that the *Leptothrix* group produces conidia within the entire thread of vegetative growth. The spiral forms of *Leptothrix* are *Gallionella* and *Spirophyllum.* The more or less incidental bending and flexing of the *Gallionella* thread does not, of course, have the same systematic significance as the regular motility of the nonflexible *Spirillum,* and we shall do well, therefore, to consider *Gallionella* as only *Leptothrix*-like. *Spirophyllum* must be regarded as the terminal form of this whole group. The threads of the latter are motile in young cells, but it is not as yet clear if the motility is flagellar or creeping. If flagellar, *Spirophyllum* would show great similarity to those organisms described by Warming, as spiral and ribbon forms of *Spiromonas;* if creeping, affinities with *Thiothrix* would be manifested.

Cladothrix (*Sphaerotilus*) is somewhat less removed from *Thiomonas* than is *Leptothrix;* but beyond the lophotrichous *Monas* forms, farther removed than *Clonothrix* and *Crenothrix,* are the corresponding non-motile coccus and sarcina forms. The correctness of considering *Clonothrix* as a member of *Crenothrix* (*Crenothrix dichotoma*), in spite of a single deviation from *Crenothrix polyspora* is that it shows false branching (as *Cladothrix dichotoma* [*Sphaerotilus dichotomus*]), which is of no great systematic significance, and is of the same order as the bunching of cells of staphylococcus, which is not considered significant. The sulfur-free thread bacteria, with the exception of spirochetes and *Cladothrix,* are iron bacteria.

The system of sulfur and thread bacteria may be constructed as follows:



The bacterial families we have considered are side branches, albeit important branches, from which yeasts and green plants arose.[16] We must consider the denitrifying bacteria as the principal branch of the bacterial system because they serve to connect the two orders to each other. The denitrifying *Monas* types are characterized chiefly by their production of bacterial fluorescein. They may or may not liquefy gelatin; the former we will designate as *Liquidomonas*, the latter *Denitromonas*.[17] The chief representative of the former is the widely distributed water organism *Bacterium fluorescens liquefaciens* (*Liquidomonas fluorescens* [*Pseudomonas fluorescens*]). As with most polar flagellated bacteria, members of these genera are not vigorous in their fermentation of amino acids or carbohydrates. They are, however, actively proteolytic and lipolytic. Evidently their nutritional requirements have always been obtained from dead animals, possibly from those types that appeared even before the plants had developed.

As in the case of the sulfur bacteria, it is believed that from these organisms both spherical and spiral forms evolved. It is difficult to know whether sulfur-free, polar flagellated cocci exist, since cocci do not usually possess poles. It is especially difficult to distinguish between lophotrichous and peritrichous cocci. Collective flagella probably arise from a point, and in such cases a coccus would undoubtedly be described as lophotrichous. Ellis, who made a special study of flagellation, believes that streptococci always possess one to three terminal flagella; and if one examines his drawings, it appears, for example, that *Streptococcus pyogenes* is peritrichous. Following the same investigations, micrococci were found either mono- or peritrichous, and sarcina always the latter. The polar flagellated, sulfur-free cocci have probably developed in two distinct directions. If this hypothetical coccus gave rise to the remaining families, these must be water bacteria further evolved toward aerobic respiration. Depending on their action on gelatin, we shall designate these cocci as *Liquidococcus* and *Solidococcus*, respectively.

We have more data on the spiral forms of the family under consideration. There are examples of all likely transitions from *Liquidomonas* to the photogenic vibrios, and the line continues only through curved rods. The vibrios are not found associated with dead animals, but are photogenic and parasitize crustacea and fish. Passing from the photogenic vibrios, we go to the pathogenic forms, such as *Vibrio*

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[16] It is evident that yeasts and algae have descended from bacteria with polar flagella, because their swarming spores are always mono- or lophotrichous.

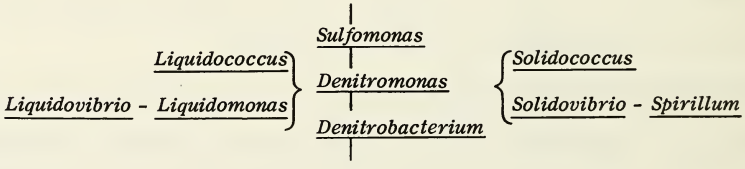
[17] If the ability of bacteria to produce proteolytic enzymes may be influenced by various factors, this would greatly influence its systematic significance.

cholerae [*Vibrio comma*], and thus traverse the entire scale from saprophytes to parasites to pathogens. With this development the ability of the latter forms to attack carbohydrates and amino acids (that is, formation of lactic acid and indol) increases. We shall give the name *Liquidovibrio* to the gelatin-liquefying vibrios. Even the members of this genus reduce nitrates to nitrites, a reminiscence of the denitrifying bacteria, but this ability is much less developed in the genus *Liquidomonas*[18] than in the genus *Denitromonas*.

It is not easy to understand the purpose of fluorescent pigment production or photogenesis. Perhaps a clue to this is that if the ability developed[19] when the earth was still dark, fluorescence and phosphorescence may have developed mutually since their producers lived in the same location. Now, following E. Wiedemann's proposition that all radiant phenomena, not arising through simple heating, be described as luminescence, we shall group all bacteria showing fluorescence or photogenesis into a family *Luminibacteriaceae*.

I consider sulfate-reducing vibrios as those spiral forms which have evolved from *Denitromonas;* reducing ability on nitrate and on sulfate must be considered to arise as separate developments. Further evolution of *Solidovibrio* brings us to the sulfur-free *Spirillum*. Important differences between monotrichous vibrios and lophotrichous spirilla do not exist—this is understandable, for whereas the shorter bacteria with incompletely developed spirality propel themselves with one flagellum, the longer, stronger, more completely developed spirals possess a tuft of flagella.

We shall place the genera *Solidovibrio* and *Spirillum* in the family *Reducibacteriaceae*. This and the family *Luminibacteriaceae* (with the inclusion of the genera *Sulfomonas* and *Denitrobacterium*) are then classified as follows:



We have now finished with the *Cephalotrichinae*.

If we now review the bacteria in this order, we see that, even as in animals, their energy is obtained almost exclusively by oxidations,

[18]Outside of *Liquidomonas pyocyanea* [*Pseudomonas pyocyanea*], one recognizes only the nonfluorescent *Liquidomonas Schirokikki*. *Centralblatt für Bakteriologie*, Section 2, II (1896), 205.

[19]It may be recalled in this connection that deep sea fish are photogenic.

and that they all, with the exception of the *Mycomycetes*, lying near *Actinomycetes* (at least at the beginning of the series), are typical water bacteria.[20] Many of the organisms of these families (see table below) grow poorly on the usual nutrient substances because the concentration of soluble organic nitrogen is too great. Endospore formation is found only in a single sulfur-free *Spirillum*, which must therefore be a quite primitive representative of these forms. A complete list of the families and genera contained in this order follows:

I. Order *Cephalotrichinae*

Family	Genus
1. *Oxydobacteriaceae*	1. *Methanomonas*
	2. *Carboxydomonas*
	3. *Hydrogenomonas*
	4. *Acetimonas*
	5. *Nitrosomonas*
	6. *Nitromonas*
	7. *Azotomonas*
2. *Actinomycetes*	1. *Rhizomonas*
	2. *Corynemonas*
	3. *Mycomonas*
	4. *Actinomyces*
3. *Thiobacteriaceae*	1. *Sulfomonas*
	2. *Thiomonas*
	3. *Thiococcus*
	4. *Thiospirillum*
4. *Rhodobacteriaceae*	1. *Rhodomonas (Chromatium)*
	2. *Rhabdomonas (Rhabdochromatium)*
	3. *Rhododictyon (Thiodictyon)*
	4. *Amoebomonas (Amoebobacter)*
	5. *Rhodothece (Thiothece)*
	6. *Rhodopolycoccus (Thiopolycoccus)*
	7. *Rhodococcus (Thiopedia)*
	8. *Lamprocystis*
	9. *Rhodocystis (Thiocystis)*
	10. *Rhodocapoa (Thiocapoa)*
	11. *Rhodosarcina (Thiosarcina)*
	12. *Rhodospirillum (Thiospirillum)*

[20]The pellicle-forming bacteria of the family *Oxidobacteriaceae* thrive only on the surface (aerobically), whereas most bacteria in the other families may also grow in the depths of a medium.

[21]The family *Trichobacteriaceae* contains so many different bacteria that it might be better to divide it into three or four families.

5. *Trichobacteriaceae*	1. *Cladothrix*
	2. *Crenothrix*
	3. *Beggiatoa*
	4. *Thiothrix*
	5. *Leptothrix*
	6. *Spirophyllum*
	7. *Spirochaete*
6. *Luminibacteriaceae*	1. *Denitromonas*
	2. *Liquidomonas*
	3. *Liquidovibrio*
	4. *Liquidococcus*
	5. *Solidococcus*
7. *Reducibacteriaceae*	1. *Solidovibrio*
	2. *Spirillum*

In the latter half of this paper, the same reasoning is applied for determining the phylogenetic relationships among the Peritrichinae. The work concludes with a diagram of a "generic tree" of the bacteria and the following paragraphs. Editor.

The development of life on earth has not proceeded in a straight line, but through the cycle of the inorganic, through plants and animals, back to the inorganic and thus to the completed forms. Plants form their constituents using the energy of the sun; animals then feed on the plants and obtain the necessary materials for building nervous tissue and brain substance, the latter necessary for generating the highest form of energy—the intellect. No limit can now be set on this development, but since there are but finite amounts of nutrients on the earth, they must be kept continually in circulation. Decomposition is therefore no less important than synthesis. In plants, autotrophic synthesis is greater than decomposition, whereas in animals, yeasts, and most bacteria the reverse is true.

Even though animals have reached the highest point of development we should not underestimate the role of bacteria and yeasts in natural decomposition processes. Microorganisms decompose vast quantities of carbohydrate and fat, and, with urobacteria, synthesize protein from the simplest materials. Further, after the death of microorganisms they are self-digested by autolytic enzymes. The decomposition of animal bodies is also work of bacteria, and it is of interest to note that the bacteria involved in the process occur in the reverse order of the natural bacteria scheme; whereas hydrolysis and fermentation begin primarily through the more recently developed organisms, complete oxidation to plant nutrients, such as carbon dioxide, nitrate, and sulfate, is brought about by the oldest bacteria. The latter also utilize

fermentation gases, such as hydrogen, carbon dioxide, and hydrocarbons in various ways, thus preventing their loss in the cycle, and bring further benefits to the plants.

The fungi are behind animals and bacteria with respect to formation of plant materials. Whereas the lowest fungi (yeasts and molds) withstand acid conditions better than bacteria, thus decomposing fruits especially well, the higher fungi attack liquefied materials, a thing bacteria cannot do well. The preference of bacteria for animals, and fungi for plants,[22] is evident, for most animal parasites are bacteria and most plant parasites are fungi. Molds utilize free organic acids better than any other living forms. This is essential in nature, since the removal of the fats not burned in the animal body is thus accomplished.

Further Readings

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[22]Since their earliest origins, fungi and plants have been closely connected, particularly in their development as suppliers of nitrogen.

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